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FINAL REPORT

19 MARCH 1963 THROUGH 19 SEPTEMBER 1964

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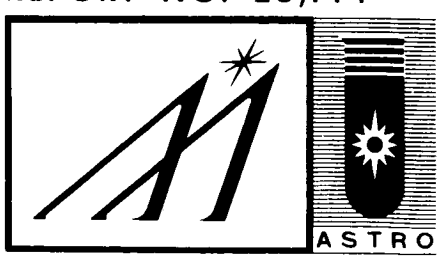

THE MARQUARDT CORPORATION

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THE Marquardt CORPORATION VAN NUYS, CALIFORNIA

Report No. 25,144

Final Report

19 March 1963 through 19 September 1964

STUDY OF BIOCHEMICAL FUEL CELLS

Contract NASw-654

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I SUMMARY

This report covers the investigations conducted during the period 19 March 1963 through 19 September 1964 in the study of biochemical fuel cells for degrading human waste.

The goals of the program were to determine the optimum conditions for degrading human waste electrochemically or, conversely, for obtaining electrochemical energy from the waste. Such a biochemical fuel cell would be intended primarily for use in space vehicles. With regard to the above goals, the investigations revealed that human waste can be electrochemically degraded in energy approximately 8%, and that anodic power densities as high as 60 milliwatts per square foot can be obtained from human waste.

The experiments conducted under this contract were designed to utilize the reactions that normally occur during the degradation of human waste, insofar as practicable, but some special treatments were evaluated (e.g., the use of anaerobic instead of aerobic conditions, adjustment of pH, control of temperature, or the addition of microorganisms not indigenous to human waste). Qualitative tests were used to determine the gross changes in the human waste, since conducting the numerous quantitative tests otherwise necessary was impractical.

Supplies of urine and feces were frozen immediately after collection, to retard biochemical reactions. The diet of an astronaut on a space mission was not known precisely, but feces supplies were obtained from volunteers on the type of diet that will probably ultimately be adopted for astronauts (i.e., a diet low in cellulose).

The experimental fuel cells were studied in both flow and nonflow systems, with platinized platinum (or platinum alloy) electrodes. Other apparatus included an impedance bridge, voltmeters, ammeters, and polarization curve plotters.

Open-circuit anodic potentials varied with experimental conditions, but potentials as large as 0.86 volt were obtained. Throughout this report, potentials are based on the saturated calomel electrode as reference.

Perhaps the most significant result of the investigation described in this report is that the electrical parameters of potential, current, and power are not constant in a biochemical fuel cell, but vary with time. These variations are caused by the metabolic reactions of the particular microorganisms present in the system, and are influenced by such things as the leakage of air into an anaerobic system, competing reactions between microorganisms , and similar factors. Therefore, definite values of the electrical parameters of a biochemical fuel cell cannot be stated when discussing certain time periods of an experiment, particularly during the first 24 to 36 hours, when the open circuit potentials vary as much as 300 millivolts.

The change of potential with time is illustrated in Figures 9, 12, 14, and 15. It is further illustrated in Table V, where a value is given for each experiment, stated as the "Open-Circuit Anodic Potential at Time of Polarization Study (volt)", and another is stated as the "Best Anodic Open-Circuit Potential (volt)". It is significant to state the potential at the time of the polarization study, since this value influences the power obtained and because it is not constant. It is also significant to state the maximum potential obtained during the experiment, because it may be possible in future experiments to stabilize the potential at the highest level. It would have been desirable, of course, to obtain the power (polarization) curves when the biochemical fuel cell was at its greatest potential, but that was not always possible because the potential varied with time and the greatest value was often obtained at night or during the weekend.

Throughout this report, values of potential, current, and power have been given whenever they were considered to be valid and stable, "stability" being arbitrarily established as fluctuating less than 20 millivolts in open-circuit potential for six hours or more. In other cases, however, no values are given for the electrical parameters; instead, only general statements are made. These general statements refer to comparisons between the potentials or power outputs obtained under various conditions, and include such statements as the fact that during certain time intervals of an experiment, more (or less) potential or power was obtained under one set of conditions than under another.

The effect of the urine:feces ratio upon the electrochemical power output of the biochemical fuel cell was measured, and it was found that there was little effect of this ratio over the range from 10 to 40 grams of feces per 100 milliliters of urine.

The addition of microorganisms or enzymes, some of which were indigenous to human waste and some of which were not, did not increase the electrochemical power output significantly. This matter is discussed in further detail in Section III.B.2.

The physical form of the waste in the fuel-anolyte was found to be a significant factor in power production; the supernatant liquid of a urine-feces mixture produced more power (0.4 milliwatts per square foot) than urine alone (0.22 milliwatts per square foot), but the standard solid-liquid mixture produced still more power (1.3 milliwatts per square foot). Diluting (with distilled water) to retard foaming decreased the power output drastically (0.33 milliwatts per square foot).

Experiments were also conducted to determine whether the physical form of the human waste during storage was also important. Freezing the urine, for storage, was convenient and was not detrimental to the power

output of the waste. Lyophilization of the feces was detrimental, in comparison to freezing; the material removed by lyophilization did not account for the loss of power. Feces from a vegetarian diet produced less power (1.6 milliwatts per square foot) than that from an omnivorous diet (as high as 5.0 milliwatts per square foot).

Experiments regarding the storage and/or pretreatment of the human waste indicated that temperature had a minor effect, over the range from 23 to 42°C; the anodic open-circuit potential varied with time because of metabolic reactions, and reached its peak value after 24 to 36 hours in the test cell (the peak anodic open-circuit potential varied, of course, with the experimental conditions); though some materials (e.g., copper) cannot be used for storing human waste because of poisoning effects on the microorganisms, it was not difficult to find suitable materials (i.e., not toxic to the microorganisms) that could be used for storing human waste, including 304 stainless steel, nickel, porcelain, glass, and Teflon-coated vessels; anaerobic conditions produced essentially the same power as aerobic conditions; pH control (below 7.0) was detrimental; and the addition of nutrients was not effective for increasing the power output.

Limited studies were made of separator materials, and cellulose acetate was chosen because it combined low electrolytic resistance with low cross contamination rates of anode and cathode chambers. Electrolytic conductivities of the appropriate solutions and separators were measured.

Polarization and power curves were obtained from the experiments that seemed to be capable of yielding such data. This was accomplished under as nearly equilibrium conditions as was practicable.

Current withdrawal or input to a test cell was maintained constant by means of a galvanostat. The withdrawal of 4.29 coulombs of current from approximately 80 milliliters of human waste represented a practical limit to the current and power output; after the withdrawal of that amount of current the anodic open-circuit potential had decreased to 0.1 volt. It also represented a practical limit to the power output, because of the limitation of current. Assuming that the 4.29 coulombs of current were obtained at an average potential of 0.6 volt, this represents a power output of 2.57 watts (0.032 watts per gram of waste).

The addition of activated sludge to a standard urine-feces mixture did not affect the electrochemical output significantly.

Literature surveys, personal communications and tests performed in this laboratory disclosed that some materials were toxic to microorganisms, but the use of these materials was easily avoided.

Studies of the electrode reactions disclosed that the cathode (non-biological, air) reaction was rate limiting by a factor of about 8 to 1. Bubbling the fuel-anolyte with inert gas provided sufficient agitation to minimize polarization.

Chemical-electrochemical reactions were separated from biochemical reactions, and it was found that the products of metabolism effectively increased the power output of the biofuel cell.

Chemical-biochemical reactions were separated from electrochemical, but there was no direct correlation between the time or amount of current passing through the flow and non-flow systems, indicating the presence of combined biochemical-electrochemical reactions that were not readily separated.

Reproducibility tests were run periodically on all systems, and the results were usually satisfactory and could be maintained within $\pm$ 0.05 volt; corrections were made in procedures and techniques where necessary to increase the level of reproducibility.

Literature surveys were made concurrently with experimentation on sewage treatment, microbiological aspects, etc.

Miscellaneous studies were conducted. In one series, it was found that it was not necessary to coat the electrodes with microorganisms. In another series, a comparison was made between methods of agitation of the electrolytes, and it was found that either the bubbling of gases (used in the nonflow systems) or pumping (used in the flow systems) was adequate for reducing polarization.

Specific enzymes and microorganisms were added to the waste, without noticeably increasing the power output of the biofuel cell.

Anodic power densities as high as 7.6 milliwatts per square foot were obtained under certain conditions. Since it was found that the cathode reaction was rate-limiting to the extent of approximately 8:1, this indicates that anodic power densities of approximately 60 milliwatts per square foot could be obtained from human waste.

Bomb calorimetric tests were made to determine the extent of degradation of the human waste. Human feces was degraded to an extent of approximately 8% of its original energy content of 5550 to (to 5100) calories per gram (lyophilized) by putting 57.3 coulombs of electricity into the waste, at potentials of -0.3 volt initially, which was increased to -1.9 volt after about two weeks; the current was maintained almost exclusively in the range from 50 to 70 microamperes.* There was no degradation due to time alone (i.e., due to metabolic reactions alone)

*7 to 10 milliamperes per square foot

over the two week period. These measurements of degradation are based upon bomb calorimetric determinations.

On the basis of the data reported herein, it seems that small amounts of power are obtainable from human waste, and that electrochemical energy can be used to degrade human waste.

II INTRODUCTION

This final report presents the results of a program performed during the period of 19 March 1963 through 19 September 1964, in the Study of Biochemical Fuel Cells, Contract No. NASw-654. The purpose of this program was to conduct empirical studies on biochemical fuel cells for degrading human waste.

Several guidelines and basic assumptions were chosen early in this program, to establish the framework within which the necessary investigations would be conducted. The guidelines and assumptions included the following:

a) The goals of the program were twofold:

   1. The optimization of the power output obtainable from a biochemical fuel cell employing human waste (urine and feces) as the fuel, and

   2. The selection of the optimum conditions for utilizing electrochemical energy to degrade human waste.

b) The primary proposed application of such a biochemical fuel cell would be in a space vehicle; this connotes requirements regarding weight, reliability, type of operation, availability of reactants, and disposal of products.

c) The various phases of investigation in this program should be designed to augment rather than to duplicate similar types of studies being conducted elsewhere. It was known that personnel at the Aeronutronics Division of Ford Motor Company (Newport Beach, California) were conducting a basic research program (NASW-655) in fundamental bioelectrochemistry, aimed at determining the biological reaction mechanism for producing electrical power via bacteria and enzyme action, and the methods of utilizing these reactions within a biological fuel cell. At the same time, personnel at Magna Corporation (Anaheim, California) were conducting studies in applied bioelectrochemistry (NASW-623), aimed at determining those microorganisms and enzymes which might be applicable to the degradation of human waste in a biochemical fuel cell and simultaneously produce electrical power.

d) The emphasis of the investigations would be on the bioanode reactions, rather than on biocathode reactions.

e) The chemical and bacteriological composition of human feces is not known in detail, and it was not possible to employ simple chemical analyses to determine the changes in composition of the feces or in the products of its degradation, caused by variations in experimental conditions. Evaluations had to be based upon qualitative rather than quantitative measurements.

f) The diet of an astronaut on a space mission, and the resultant composition of his feces, are not known precisely. However, it was assumed that such a diet would contain less bulk (indigestible matter) than that of a person on earth. This factor was considered in collecting feces for the experiments conducted during this program.

g) In an effort to standardize the test conditions insofar as possible, in order to ensure a high level of reproducibility and permit study of the effects of single variables, a set of experimental conditions was chosen. These conditions are described in Table I, for both flowing and non-flow systems. The conditions described in Table I were used for the experiments described in this report, unless otherwise stated. The test cells are shown in Figures 1 through 6.

III DISCUSSION

A. APPARATUS AND EQUIPMENT

The static system is illustrated in Figure 1. The figure illustrates a glass H-cell, consisting of tubing about 7-3/4 inches long by 1-1/8 inch I.D. and has a horizontal distance of 2-1/2 inches between vertical tubes, and a course-fritted glass disc constructed in place. The electrodes are platinized platinum foil, with 1 square inch area on each side, approximately 3-1/2 inches apart. An agar plug pulled into the fritted glass disc by vacuum forms the electrolyte separator. Also illustrated are gas bubblers for bubbling gaseous helium and oxygen (or air) over the anode and cathode, respectively. A Luggin capillary (salt bridge) is shown, to provide measurements of potentials of one electrode (the anode) versus a reference electrode (saturated calomel).

Another type of static cell used in screening experiments is illustrated in Figure 2. This cell is similar to that shown in Figure 1, with the exception that the two halves of the cell are joined with O-ring joints, which are held together with a clamp. The O-ring joints permit the evaluation of separators other than agar. A cellulose acetate separator is illustrated; however, ion exchange or dialysis membranes may also be used. Figure 2 illustrates a cell with a mixture of human feces-urine at the anode and a non-biological (oxygen) cathode.

Many static cells may be used in screening experiments, as illustrated in Figure 3. The rack will accommodate up to 18 cells, so that comparisons of several separator and electrode materials may be conducted simultaneously. The digital printout box in the background will accommodate up to 50 cells, reading voltage or current at prearranged time intervals.

At the top of the rack in Figure 3, another type of glass cell may be observed. It is 4-1/4 inches long, and is constructed of 2-inch O.D. glass tubing. This cell provides larger electrode surface, and the electrodes can be placed closer together (within one-half inch of each other) than in the glass H-cells previously described.

At the lower left of Figure 3, an oxygen meter (Beckman Model OM-1) may be observed; the electrode is in the right arm of the second H-cell (measured from the left). This electrode measures the amount of oxygen in the solution, and is used to establish aerobic or anaerobic conditions required in the cells.

A continuous flow system is illustrated in Figure 4. Two plastic cells are illustrated, connected so that the anolyte and catholyte flasks are common to both. The 5-neck flasks provide for

gas bubblers, gas release tube, pH adjustment, nutrient addition, withdrawal of media to be pumped through the cells and returned to the 5-neck reservoirs, and stirring by means of magnetic stirrers. The cells are constructed of acrylic plastic (Lucite), and the tubing of Tygon. The pumps are located in the center of the photograph. The cells may be clamped-off from each other when taking electrical measurements. In the foreground are a voltmeter, an ammeter, and a decade resistance box.

A schematic drawing of the continuous flow system is shown in Figure 5. The drawing is liberally labelled, so no further description is provided.

A dismantled plastic cell is illustrated in Figure 6. From the left in the photograph, are shown a solid end plate, a screen electrode with water repellent and chemically resistant paint to prevent leakage, two electrode reservoirs (one for anolyte and one for catholyte), a second screen electrode, and a second end plate, respectively. A separator, such as a cellulose acetate or ion exchange membrane (not shown) would be placed between the electrolyte reservoirs. The tubes on opposite sides of the reservoirs provide flow into and out of the cell, while threaded nylon rods are passed through the holes at each corner to hold the cells in place. The plastic cell is 3 inches square and has electrodes 1-5/16 inches apart, with an exposed diameter of 2-1/8 inches. It is constructed of 80-mesh platinized platinum screen.

An impedance bridge (Universal Model 291) is illustrated in Figure 7. By means of this bridge, both resistance and capacitance can be balanced when measuring electrolytic conductivities and resistances of the many electrolytic solutions and separators currently studied in this program.

A 50-channel digital voltmeter is illustrated in Figure 8, which provides either printout or a graph on an X-Y plotter. The 50-pin plug-in box is illustrated in the insert.

An X-Y polarization curve plotter is illustrated in the center of Figure 8. The polarization curve plotter is described in detail in Section III F of this report.

B. FUEL COMPOSITION

Four experiments were conducted during this program to determine the proper fuel-anolyte for a biochemical fuel cell employing human waste. The experiments were designed to furnish the following general types of information:

1. Optimum composition or feces:urine-ratio, for power output;

2. Effect of addition of microorganisms or enzymes, indigenous or non-indigenous to human waste;

3. Physical form of fuel-anolyte; i.e., liquid only, or a liquid-solid mixture;

4. Desirable physical state of the waste during storage; and

5. Effects of diet and physiological variations upon the electrochemical characteristics of human waste.

These experiments will be discussed in more detail in the following paragraphs.

1. Optimum Concentration or Feces:Urine Ratio:

The composition of the fuel-anolyte was varied from urine alone to feces alone, with various intermediate ratios of urine and feces. Ideally, the ratio in which human waste is eliminated would be desirable for use in the biofuel cell. The amounts of urine and feces eliminated as a result of normal diets are given in Tables II and III, Appendix A.

The data given in the tables show that the output of both feces and urine vary considerably, depending upon the diet, and therefore this ratio can vary from 0.03 to 0.37.

This ratio was not used in these studies, however, because even though the diet of an astronaut on a space mission is not known, the ratio of urine to feces eliminated by an astronaut would probably be higher than that implied in the values stated in Tables II and III, which are based upon the average elimination of people on earth. The diet of an astronaut probably would contain less bulk (non-digestible matter), and less solids would be eliminated.

This factor was considered when obtaining the supply of feces for the experiments conducted under this contract. The feces were obtained from volunteers on a low-cellulose diet.

The proximate analysis of human feces is given in Table II of Appendix A. The analysis of urine is given in Table III, same Appendix.

The results of the experiments conducted to determine the effect of the urine:feces ratio in the fuel-anolyte are summarized in Table IV, Appendix A. Though the data have been arranged in Table IV in the order of increasing feces content of the fuel to facilitate comparison, it should be noted that these tests encompassed two experiments, at different times, rather than a single experiment; this explains the choice of concentrations. The data obtained from Experiment 8 seemed to indicate that the electrochemical power output increased with increasing feces content of the fuel even at the level of 25 grams feces in 100 milliliters of urine. Later information, obtained in Experiment 9, indicated that a peak in power output exists at concentrations between 10 and 20 grams feces per 100 milliliters of urine.

The data obtained at 15 grams feces per 100 milliliters of urine are considered to be in error. The reason for the apparently erroneous data, as well as the poor reproducibility of data obtained from the two samples at a concentration of 10 grams feces per 100 milliliters of urine, is that the electrochemical characteristics of these biofuel cells vary considerably with time. An illustration of this phenomenon may be seen in Figure 9. The variation of open-circuit potential with time was obtained with the data readout system shown in Figure 8, which is described in further detail in Section A, Apparatus and Equipment.

An interesting aspect of this study was the reversal of the anodic open-circuit potential with time. The order of potentials early in the experiment was reversed later in the experiment; e.g., after 20 hours, the anodic potentials increased with increasing feces concentration whereas after 62 hours, the anodic potentials decreased with increasing feces concentration.

An experiment was later conducted to verify the previous results obtained in determining the effects of feces-urine ratio on the electrochemical characteristics of the biofuel cell. The results of this experiment (No. 43) are presented in Table V. The cells are identified as follows:

Cell Number	Gms. Feces/100 ml. Urine
I	10
II	20
III	30
IV	40

It may be concluded on the basis of these data that the feces-urine ratio has a relatively minor effect upon the electrochemical characteristics of the biochemical fuel cell, over the range from 10 to 40 grams of feces per 100 milliliters of urine.

An experiment was run to determine the electrochemical properties of feces without urine. The conditions of the experiment were the same as those for the non-flow system described in Table I of the Appendix, except that deionized, distilled water was used instead of urine. The data indicated that the power output from feces alone was negligible (see Table V, Appendix A, Experiment 66) although it had been found that feces was effective when added to urine. (See Table V, Appendix A, Experiment 8.)

2. Effect of Addition of Microorganisms or Enzymes, Indigenous or Non-Indigenous to Human Waste:

The addition of microorganisms or enzymes (e.g., Escherichia coli, Pseudomonas denitrificans, cellulase) to human waste did not increase the power output significantly.

Cellulase was added to feces in an effort to decompose the cellulose, but without noticeable effect (see Table V, Appendix A, Experiment 66. The fuel-anolyte mixture contained 5 grams cellulase and 30 grams feces in 100 milliliters of sterile, distilled, deionized water.

Polarization and power data were obtained from a fuel-anolyte containing Escherichia coli, which had been added to a feces-urine mixture. The E. coli suspension in brain-heart infusion was centrifuged at 5000 rpm for 20 minutes; the supernatant liquid was removed, and the E. coli was resuspended in 1 milliliter sterile, deionized water. The resuspended E. coli was added to the feces-urine mixture prepared as described in Table I, Appendix A. The peak total power density was 9.0 milliwatts per square foot and the anodic power density 1.2 milliwatts per square foot; the short-circuit density was 25 milliamperes per square foot. (See Table V, Appendix A, Experiment 6.)

In another experiment, the effect of the addition of E. coli to the supernatant liquid of a feces-urine mixture was determined. A feces-urine mixture was made as described in Table I, Appendix A. The mixture was centrifuged at 5,000 rpm for 20 minutes at 15°C. A portion (90 milliliters) of the supernatant liquid was mixed with 10 milliliters of an inoculum of E. coli. The inoculum had been prepared by growing 1/2 milliliter of E. coli culture in 500 milliliters of brain heart infusion. The peak anodic power density was 4.0 milliwatts per square foot and the peak total power density 9.2 milliwatts per square foot (see Table V, Appendix A, Experiment 4).

3. Physical Form of Fuel-Anolyte (Liquid Only, or Liquid-Solid Mixture):

a. Comparison of Urine with the Supernatant Liquid from a Feces-Urine Mixture:

A test was conducted to compare the electrochemical power output of urine with the supernatant liquid obtained by centrifuging a feces-urine mixture, prepared as described in Table I, at 5000 revolutions per minute for 20 minutes. The data are compared below:

Description	Urine	Supernatant-Liquid of a Urine-Feces Mixture
pH, Initial	5.7	6.6
pH, Final	8.9	8.8
Best anodic open-circuit potential (volt)*	-0.545	-0.687
Anodic open-circuit potential at time of polarization study (volt)*	**	-0.603
Peak anodic power density (mw./sq.ft.)	**	0.06
Anodic potential at peak power density (volt)*	**	0.08
Current density at peak power (ma/sq.ft.)	**	7.5
Short-circuit current density at time of polarization study (ma./sq.ft.)	**	19

*All potentials are referred to saturated calomel electrode
**Current was so low that the power was insignificant.

Insufficient current was obtained from the urine biofuel alone to permit a polarization study. The initial concentration of the urine-feces mixture was 30 grams feces in 100 milliliters urine.

b. Standard Concentration of Urine and Feces:

Polarization and power curves were obtained with the flowing system, as shown in Figures 10 and 11. The fuel-anolyte was 30 grams human feces in 100 milliliters fresh, human urine (Table I, Appendix A). A peak anodic power density of approximately 1.3 milliwatts per square foot was obtained (see Table V, Appendix A, Experiment 1).

c. Urine-Feces Diluted:

A mixture was made of 30 grams human feces in 100 milliliters fresh, human urine, as above. The mixture was homogenized and then diluted (10%) with sterile, deionized water to retard foaming. The peak anodic power density was 0.33 milliwatts per square foot, and that of the total cell was 0.59 milliwatts per square foot (see Table V, Appendix A, Experiment 2).

d. Supernatant Liquid Diluted:

A mixture was made of 30 grams human feces in 100 milliliters fresh, human urine, as above. After mixing and homogenization, the material was centrifuged at 5000 rpm. for 20 minutes. The supernatant liquid was diluted 1:1 with sterile, deionized water to determine the effectiveness of this concentration as a biofuel. A peak anodic power density of 0.15 and a total cell power density of 0.22 milliwatts per square foot were obtained (see Table V, Appendix A, Experiment 3).

4. Desirable Physical State of the Waste During Storage:

a. Comparison of Fresh Urine and Frozen-Reheated Urine:

The human urine used in these investigations was usually frozen and later reheated, for simplicity in procurement. However, after freezing, some of the solids did not go back into solution, even after heating to 120°F; heating to higher temperatures was avoided because of the possibility of degrading biological components of the waste.

Earlier measurements of the effects of these solids upon the electrolytic conductivity of the urine showed that the electrolytic conductivity of the urine was significantly less when these solids were formed and were not redissolved. (See Table VII)

An experiment was run to determine whether removing these solids from the urine also affected the electrochemical power output of the fuel. There seemed to be little difference in the power output of the two types of urine (fresh or frozen and thawed). The data are reported in Table V, Appendix A, Experiment 70.

The power output from the frozen and thawed urine was lower than that obtained previously from similar systems. The data obtained with the fresh urine seemed to have been obtained at an inopportune time, since the anodic open-circuit potential at that time was -0.450 volt and later (after 48 hours) had attained -0.643 volt.

The previous procedure of freezing and thawing the urine, for convenience, was continued.

b. Lyophilized Frozen and Fresh Frozen Feces:

For storage purposes, the lyophilization of feces may be desirable if the biochemical and electrochemical activities of the feces are not affected detrimentally. An experiment (Experiment 5) was conducted to compare the biofuel characteristics of lyophilized feces with those of

fresh frozen feces. Two H-cells were assembled as described in Table I, Appendix A; two more were assembled in which lyophilized feces was used. The concentration of lyophilized feces was 8 grams in 100 milliliters urine, which corresponds to the concentration of 30 grams of whole feces in 100 milliliters urine used in the two cells described above (whole feces is approximately 75% water by weight). The feces was lyophilized after homogenizing in distilled water; lyophilization from a feces-urine mixture was not successful.

The open-circuit potential of the lyophilized material varied from +0.100 volt (saturated calomel electrode reference) to -0.625 volt over a period of 70 hours, and approached that of the fresh frozen feces; however, this value was attained by the fresh frozen feces after only about 5 hours.

c. Fresh and Lyophilized Fresh Feces:

An experiment (Experiment 7) was conducted to compare the biofuel value of fresh feces with that of lyophilized fresh feces. The fresh feces fuel-anolyte was prepared as described in Table I, Appendix A. The lyophilized fresh feces was initially prepared in a similar manner, then lyophilized as described in Section III.B.4.b; the weight of lyophilized feces used in the experiment was 8 grams, corresponding to 30 grams of whole feces.

The peak anodic power density of the lyophilized fresh feces-urine was 0.21 milliwatts per square foot and the peak total power density 0.42 milliwatts per square foot; the peak anodic power density of the fresh feces-urine was 1.5 milliwatts per square foot.

Because of the reduction in power of the lyophilized feces as compared to the fresh feces, an experiment (Experiment 12) was run to determine whether any of the material removed during lyophilization is electrochemically active. The material removed while lyophilizing feces in a concentration of 30 grams fresh feces in 100 milliliters distilled water was thawed and used as a fuel-anolyte. This system displayed an anodic open-circuit potential of -0.146 volt (versus saturated calomel) as its best value, but insufficient current was obtained to make a polarization plot. Therefore, the reduction in power was not due to electrochemically active substances removed from the frozen lyophilized feces.

Lyophilized feces was obtained from another company conducting similar types of research (see Section II, Introduction); the feces was lyophilized in a commercial freeze drier. The lyophilized feces was then mixed with urine, as described for the study of lyophi-

lized material in Section III.B.4.b, to provide comparable data. Duplicate tests were run (Experiment 14). The initial pH of this mixture was 6.7; the best anodic open-circuit potential was -0.641 volt; the peak anodic power density was 4.1 milliwatts per square foot, at an anodic open-circuit potential of -0.600 volt; and a short-circuit current density of 72 milliamperes per square foot was obtained. The reproducibility of this system is shown in Figure 9.

5. Effects of Diet and Physiological Variations upon the Electrochemical Characteristics of Human Waste:

It was known that diet and physiological variations would affect the composition and properties of human waste. Though it was not practicable to measure the effects of small variations, an experiment was conducted to measure the effects of large variations in type of diet.

In this experiment, the fuel-anolyte mixture was prepared as described in Table I, Appendix A, but the feces from a vegetarian was substituted for feces from an omnivorous diet. The peak anodic power density was 1.6 milliwatts per square foot, for the vegetarian feces (see Table V, Appendix A, Experiment 10), while it ranged as high as approximately 5 milliwatts per square foot for omnivorous feces (see Table V, Appendix A).

C. PRETREATMENT OF HUMAN WASTE

1. Effects of Temperature, Time, Vessel Material, Composition, Atmospheric Conditions, and pH Control:

Some studies were conducted on the effects of pretreatment of human waste to increase its electrochemical activity, by promoting different reactions and greater potentials, as well as faster reactions and increased current density. Other reasons for pretreating the waste might be to (1) increase the rate of degradation of the waste, or (2) alter the products formed, particularly gases which might leak into the cabin of a space vehicle. The possibility remains that no pretreatment will be desirable, because of the attendant loss of energy.

The pretreatment of human waste has included investigations of the relatively simple variables of temperature (23° to 42°C), time (1 to 9 days), vessel material (glass or type 304 stainless steel), composition of human waste (i.e., feces:urine ratio), atmospheric conditions (aerobic or anaerobic), and pH control. The following general results were obtained:

a. The effects of temperature upon the electrical parameters were not consistent. This was doubtless due to the fact that metabolic variations obscured the slight variations in potential due to temperature change.

b. There was a general increase in open-circuit anodic potential with time for the first 24 to 36 hours, followed by a slow decrease to a pseudo-plateau after an additional 1 to 7 days. The power curves could not be taken at all time intervals, but the increase in potential during the initial 24 to 36 hours of an experiment implies an accompanying increase in current and power (for a given system).

c. Possible inhibitory effects of the stainless steel were evaluated; none were found. Various materials (Teflon, nickel, porcelain, etc.) could be used as liners for the metal storage vessels, if desired, although they would not be necessary if vessel materials such as type 304 stainless steel were used.

d. Anaerobic conditions were essentially the same as aerobic. From the viewpoint of waste degradation, it might be desirable to employ successive stages of aerobic-anaerobic processes.

e. The pH control (below 7.0) was detrimental. The pH was not usually controlled, and increased as high as 8.8 from an initial pH of 6.1 to 7.1.

f. The addition of nutrients to the bulk solution was not effective in increasing the electrochemical power output of the human waste.

One experiment was run to determine the effects of temperature alone as a pretreatment. Indigenous microorganisms were used; i.e., the fuel-anolyte was not sterilized for this experiment. The results are presented in Table V, Experiment 51. The data indicated that when indigenous microorganisms are used, better electrochemical characteristics are obtained if the system is maintained at room temperature than if it is incubated at 95-100°F.

Two experiments were conducted to determine the electrochemical activity of human waste mixtures to which selected microorganisms had been added. The conditions of the experiments were essentially the same as those outlined for the non-flow system in Table I of Appendix A, except that the feces-urine mixture was sterilized of indigenous microorganisms, by autoclaving at 250°F for 20 minutes.

In one experiment, 1% of a culture of Escherichia coli in nutrient broth was added to the sterile feces-urine fuel-anolyte mixture. Two cells were thus assembled, one maintained at room temperature (approximately 75°F) and the other incubated at 95-100°F (essentially body temperature).

During the first 85 hours of this experiment, the anodic open-circuit potentials of the two cells were essentially the same, although they reached a minimum of -180 millivolts and a maximum of -850 millivolts. After that time, the anodic open-circuit potential of the cell maintained at room temperature was 150 to 250 millivolts better than that of the incubated system, which decreased slowly from -0.650 volt to about -0.500 volt over the next 215 hours. The total time of the experiment was 350 hours. Polarization and power data for this experiment (No. 57) are summarized in Table V of Appendix A.

In another experiment, conducted in a manner similar to that described above, a 1% culture of Clostridium sporogenes in nutrient broth was added to the sterilized fuel-anolyte instead of the E. coli. Again, two cells were assembled; one was maintained at room temperature (75°F) and the other was incubated (95-100°F).

The anodic open-circuit potential of the incubated cell was approximately 250 millivolts better than that of the cell at room temperature, although both changed with time because of metabolic reactions, for the period from 15 to 57 hours, then they became essentially the same (approximately -0.475 volt) for the next 94 hours. Polarization and power data for this experiment (No. 58) are summarized in Table V of Appendix A.

On the basis of these experiments, there seems to be no significant effect of raising the reaction temperature from room temperature to a standard incubation temperature, and the microbes described above were not as effective as indigenous microorganisms in producing electrical power.

The selection of the particular microorganisms was based upon the fact that E. coli is indigenous to human waste, but it is only one of the various types of microorganisms indigenous to human waste. By first autoclaving the waste and then preferentially adding E. coli back into it, there was an attempt to avoid competing reactions between E. coli and other indigenous microorganisms, as well as avoiding the production of products by the metabolism of some microorganisms which may be poisonous or inhibitory to E. coli. The Clostridium was chosen because it is also indigenous to man and it is an obligate anaerobe, unlike E. coli, and it was thought that conditions at the bio-anode of the fuel cell would be essentially anaerobic.

2. Addition of Nutrients:

A few experiments were conducted, in which nutrients were added to the human waste to promote bacteriological growth and metabolism. These experiments are described in detail in the following paragraphs. All readings were taken at room temperature, thus avoiding temperature effects on electrical properties. Results of these experiments are presented in Table VI.

P-1. Fuel-anolyte: 10 percent (by weight) feces, 10 percent urine (both non-sterile), and 80 percent triple filtered sea water (final filtration with 0.45 μ Millipore paper); homogenized. Catholyte: non-biological (air), triple filtered sea water. Electrodes: platinized Pt foil, 1 square inch on each side, both sides exposed. Separator: agar plug. Temperature: 99°F. Storage vessel: stainless steel (type 304).

P-2. Fuel-anolyte: 5 percent (by weight) feces and 5 percent urine (both non-sterile), with 90 percent sterile sulfate medium (see Table XIII), homogenized. Catholyte: non-biological (air), with sterile sulfate medium. Electrodes: platinized Pt foil, 1 square inch on each side, both sides exposed. Separator: agar plug. Temperature: 99°F. Storage vessel: stainless steel (type 304). pH adjusted to 6.5.

P-3. Fuel-anolyte: 5 percent (by weight) feces and 5 percent urine (both non-sterile), with 90 percent sterile sulfate medium (see Table XIII), homogenized. Catholyte: non-biological (air), with

sterile sulfate medium. Electrodes: platinized Pt foil, 1 square inch on each side, both sides exposed. Separator: agar plug. Temperature: 108°F. Storage vessel: stainless steel (type 304). pH adjusted to 6.0, and air was bubbled continuously into the storage vessel.

P-4. Fuel-anolyte: 5 percent (by weight) feces (non-sterile) and 95 percent sterile sulfate medium (see Table XIII), homogenized. Catholyte: non-biological (air), with sterile sulfate medium. Electrodes: platinized Pt foil, 1 square inch on each side, both sides exposed. Separator: agar plug. Temperature: 99°F. Storage vessel: stainless steel (type 304). Air was bubbled continuously into the storage vessel.

P-5. Fuel-anolyte: 5 percent (by weight) feces and 5 percent urine (both non-sterile), with 90 percent sterile sulfate medium (see Table XIII), homogenized. Catholyte: non-biological (air), with sterile sulfate medium. Electrodes: platinized Pt foil, 1 square inch on each side, both sides exposed. Separator: agar plug.

a. Temperature: 73°F. Storage vessel: glass H-cell.
b. Temperature: 104°F. Storage vessel: glass H-cell.
c. Temperature: 104°F. Storage vessel: stainless steel (type 304)

P-6. Fuel-anolyte: 5 percent (by weight) feces and 5 percent urine (both non-sterile), in 90 percent sterile sulfate medium (see Table XIII), homogenized. Catholyte: non-biological (air), with sterile sulfate medium. Electrodes: platinized Pt foil, 1 square inch on each side, both sides exposed. Separator: agar plug. Temperature 73°F. (room temperature).

a. Storage vessel: glass H-cell.
b. Storage vessel: stainless steel (type 304)

P-7. Fuel-anolyte: 30 grams of feces in 100 grams of urine (both non-sterile). Catholyte: non-biological (air), in aqueous salt solution (5 percent NaCl - 5 percent KCl by weight). Electrodes: platinized Pt foil, 1 square inch on each side, both sides exposed. Separator: cellulose acetate. Temperature: room temperature (73°F.) Storage vessel: glass bottle.

D. SEPARATOR MATERIALS

No extensive studies were made of separator materials, although such studies were within the scope of the contract, because it was agreed that the investigations of other parameters affecting the operating characteristics of the biofuel cell were of more fundamental importance. Studies were limited to one anion exchange membrane, one cation exchange membrane, one type of cellulose acetate, and agar plugs. The cellulose acetate separators seemed to be reasonably satisfactory, based upon low electrolytic resistance and low contamination rate of anode and cathode chambers, either by microorganisms or chemical substances.

E. ELECTROLYTIC CONDUCTIVITY

1. Electrolytes

The impedance bridge and conductivity cell shown in Figure 7 and described in Section A were used to determine the cell constants of the various cells, and to measure the electrolytic conductivities of the solutions and separators used under this contract. Some typical resistances of solutions obtained at a frequency of 400 cycles per second are tabulated below. Detailed data are presented in Table VII.

Substance	Electrolytic Resistance (ohms)
Deionized water	exceeds 2×10^6
Saturated KCl	3.1
0.1 N KCl	148.8
5% by wt. NaCl + 5% KCl	13.24
Urine, fresh	59.81
Urine, frozen and thawed*	92.2

The only electrolyte described above which was not used in the investigations under this contract was the urine which was frozen and thawed (not reheated). The purpose of measuring the electrolytic resistance of the frozen and thawed urine was to determine the effect of the removal of certain precipitates or agglomerates, formed during freezing, upon the electrolytic resistance of urine. Although fresh urine was used in some of the biofuel cell experiments, the urine was usually frozen and stored for convenience. The frozen urine was not only thawed before use, but was heated gently (120°F) to put the solid particles back into solution; an entire container of urine was thawed and mixed thoroughly before a portion was withdrawn. The removal of the solid particles from urine reduced the electrolytic conductivity of the solution. The temperature to which the urine was reheated was not high enough to be detrimental to or to decompose the constituents of urine.

2. Separator Materials

Measurements were also made of the electrolytic resistances of various types of separator materials. The investigation was not intended to be extensive, and was limited to one anion exchange membrane, one cation exchange membrane, one dialysis membrane, and one form of cellulose acetate. The specific resistivities of the separators are summarized below, and detailed data are presented in Table VIII.

*Thawed at room temperature; not reheated.

Separator	Specific Resistivity (ohm-cm.)
Anion exchange membrane	33.6
Cation exchange membrane	47.2
Dialysis membrane	79.6

Only erratic results were obtained when the resistivity of the cellulose acetate was measured.

Measurements of the resistivities of the separators were made in the conductivity cell shown in Figure 7; the cell had an internal diameter of 40 millimeters and the distance between electrodes was approximately 10 inches.

Another property of separators that is of primary importance in the biochemical fuel cell, and which was also evaluated in this laboratory, is the ability of the separator to retard the contamination of the electrolytes caused by mass diffusion through the membrane, both chemically and bacteriologically. On this basis, the dialysis membrane above and listed in Table VIII was not used in these studies; although it presented a relatively low electrolytic resistance, it was found to allow gross contamination by diffusion in a relatively short period of time.

Cell constants were obtained, using an oscilloscope (Tektronix Type 502 Dual Beam) with the impedance bridge shown in Figure 7. The electrolyte used was saturated potassium chloride in deionized water. Detailed data are given in Table IX, and sample calculations are presented in Appendix B.

F. POLARIZATION AND POWER CURVES

Some typical polarization and power curves are illustrated in Figures 10 and 11. The polarization curves (voltage vs. current) were plotted with an X-Y plotter (Moseley Autograf), modified in this laboratory to produce a decreasing resistance in the external circuit of the cell of approximately 33,000 ohms per hour (i.e., from 200,000 ohms to short-circuit in approximately 6 hours). After obtaining the potential reading at open-circuit, the initial reading under load was at an external resistance of 200,000 ohms. At short-circuit, the resistance in the external circuit was less than 1 ohm. The total time of 6 hours to obtain the polarization curves was chosen in order to provide as nearly equilibrium conditions as feasible in a changing system, and at the same time, be commensurate with a day's operation in the laboratory.

The power curves were obtained by cross-plotting the potential vs. current data from the polarization curves.

Polarization and power data obtained from other experiments conducted during this program have been summarized where applicable, and are presented in Table V, Appendix A.

G. CONTROL OF CURRENT BY GALVANOSTAT

A galvanostat-potentiostat, constructed in this laboratory, was used in some experiments to continuously control the current, either withdrawn from or fed into a biofuel cell, at a constant value. The purpose of the experiments was to determine the effect of current withdrawal or introduction upon the electrochemical properties of a biofuel cell and to determine the total amount of electrochemical energy obtainable from human waste, or the extent of degradation of human waste accomplished by the introduction of electrochemical energy.

The experiments were conducted in both the flow and nonflow systems, under the conditions described in Table I.

1. Withdrawal of Current from Biochemical Fuel Cell:

In one experiment there was no noticeable effect of current withdrawal (at a rate of 1 milliampere) on the anodic potential, after 32 hours. The current of 1 milliampere represents a current density of approximately 40 milliamperes per square foot.

In another experiment, a current of 18 to 34 microamperes was withdrawn from the system, after periodically resetting the galvanostat; this represents a current density of 2.6 to 4.9 milliamperes per square foot. Control of current withdrawal with the galvanostat was maintained within 1 microampere. Over a period of ten days, a total of 4.29 coulombs was withdrawn from the cell. The open-circuit anodic potential improved with time, from an initial value of -0.43 volt to -0.66 volt (referenced to saturated calomel).

The portions of the change caused by time alone (i.e., metabolic) and that due to electrochemical reactions resulting from current withdrawal could not be separated, since both occur simultaneously in the non-flow system (this difficulty was resolved in subsequent experiments by simultaneously running a control cell with each test cell.) Each control cell (through which no current flowed) provided data regarding the effect of time alone on the properties of the waste, while each test cell provided data on the combined effects of time and current. By this method, the effects of current were determined.

The withdrawal of only 4.29 coulombs of current was not limited by the fuel cell reactions; the experiment could have been continued, but a polarization run was made on this system after the 4.29 coulombs of current had been withdrawn, and the electrical power output was extremely poor. The data are summarized in Table V (Experiment 47), where it is seen that the peak anodic power density was only 0.2 milliwatts per square foot (at 0.1 volt and 1 milliamperes per square foot) and the short circuit current density only 4 milliamperes per square foot at that time.

2. Use of Electrochemical Energy to Degrade Human Waste:

A potential of approximately 1.2 volt was imposed in series on the biofuel cell to increase the current flow and the resulting electrochemical reactions occurring over a given time period. The current flow varied from 50 to 70 microamperes, the total time elapsed was 20 days, and the total current was 57.3 coulombs. A control cell was assembled, using a portion of the same fuel-anolyte mixture as that in the cell that had the imposed potential (Experiment 63). Data obtained from bomb calorimetric experiments are described in Section III.P.

It is impractical to make a detailed chemical analysis of the human waste before and after experiment to determine the effect of the imposed potential, because of the complex nature of the material. The most expeditious alternative is to make bomb calorimetric determinations of the heating values, after lyophilization, of feces-urine mixtures from (a) the original sample, (b) the control cell, and (c) the cell subjected to the electrical current. Bomb calorimetric data are useful in determining the change in energy content of the fuel, and its resultant state of degradation.

In another Experiment (No. 74), two nonflow cells were assembled, under essentially the same conditions described in Table I, except that the fuel-anolyte of one cell was bubbled with oxygen instead of helium. The cells were connected in series to a galvanostat and a current of 10-14 microamperes was put into the cell over a period of 15 days, for a total of 14.14 coulombs. The area of each electrode was 1 sq. in. Bomb calorimetric data provide data for comparing the relative effects of aerobic and anaerobic conditions in degrading human waste.

The relative effects of aerobic and anaerobic conditions upon the composition of the fuel, without current flowing into the cell, is described in Section III.H.

The combined effects of the use of electrochemical energy and addition of activated sludge to human waste was also determined. The experimental conditions were essentially the same as those described in Table I, except that activated sludge was added to the feces-urine mixture in a final volume ratio of 1 volume of activated sludge to 9 volumes of the usual urine-feces mixture, and the fuel-anolyte was maintained aerated (Experiment 80).

H. EFFECT OF AEROBIC CONDITIONS IN THE FUEL-ANOLYTE

The final phase of this contract was to be devoted to some extent to studies of the use of electrochemical energy in degrading human waste, in addition to maximizing the electrochemical power output of human waste as a biofuel as had been done in the earlier phases of this program.

In an attempt to facilitate the degradation process, an experiment was conducted to determine the effect of aerobic conditions upon the electrochemical power output of the biofuel cell, in contrast to the anaerobic conditions used in the past. The conditions of the experiment were generally the same as those which were selected in the early phases of this program in an effort to standardize the experiments and minimize the variables. These conditions are summarized in Table I. Two cells were assembled. One was a control, assembled as described in Table I; the other was a test cell, in which oxygen instead of helium was bubbled through the fuel-anolyte. The fuel-anolytes and catholytes used in both cells came from the same mixtures. The results of the experiment (Experiment 67) are summarized in Table V.

It was found that there was essentially no difference in maintaining the fuel-anolyte under aerobic as contrasted to anaerobic conditions. The open-circuit anodic potentials and short-circuit current densities of both systems improved during the first 100 hours of the test, due to metabolic reactions. However, at any given time there was only a negligible difference in open-circuit anodic potentials and short-circuit current densities between the aerobic and anaerobic systems.

I. ACTIVATED SLUDGE

A sample of activated sludge was obtained from the local sewage treating plant (courtesy Mr. Joseph Nagano, Hyperion Sewage Works, Playa del Rey, California). This material was maintained in an aerated, viable condition by bubbling with gaseous oxygen and by periodically adding fresh human waste to the sludge. This material was added to human waste prepared as described in Table I, but without noticeable effect on the electrochemical power output.

J. MATERIALS EVALUATION AND TESTING

The possibility of problems regarding the compatibility of materials used in the various experimental systems was recognized early in this program, and a continuing effort was made to eliminate materials which might exert inhibitory effects on the electrical power characteristics of the biofuel cell, either by their deleterious effects on the microorganisms or by physically poisoning the electrodes. Answers to these questions were sought in the literature, by means of tests conducted in the laboratory, and by requesting that the co-contractors and all other personnel associated with this program forward any pertinent information to this laboratory, regarding the effects of materials on microorganisms.

The results were encouraging, and there was no need for major revisions in the types of materials used in these studies. However, the literature survey and inquiries were profitable. Personnel from the NASA office quoted references to investigations which demonstrated a poisoning effect caused by silicone grease deposited on electrodes; no such stopcock grease was used in these studies.

The literature survey indicated some toxic effects of metals and plastics upon bacteria. It was found that copper, zinc, brass, and butyl rubber were toxic; while tin, lead, aluminum, stainless steel, epoxy resin, polyethylene, silicone rubber, and vinyl were not.

Although much valuable information was obtained from the literature survey and inquiries, not all of the questions regarding the possible effects of materials on microorganisms could be answered, of course. Therefore, experiments were conducted in this laboratory, where necessary. The materials testing was a continuing aspect of this program; all new materials introduced into the program were tested before being used routinely.

The experiments were conducted by placing samples of sterilized materials on sterile trypticase soy agar in sterile Petri dishes. The test preparations were stored in the refrigerator for ten days, to allow diffusion of toxic products into the medium, and then inoculated with the appropriate microorganism (e.g., *E. coli*). At the end of one experiment, there was no effect of types 304 or 321 stainless steel, silicone rubber (Dow Corning S-7180), Kimble R-6 and Pyrex glasses, epoxy cement (cured Epocast H-1368, Furane Plastic), Tygon plastic tubing (type R-3603), Plexiglas, chemically resistant paint (Temprotec TP 220 Red, Ryan Herco Products Co., Burbank, Calif.), rubber and Neoprene stoppers, cellulose acetate separator (E. H. Sargent Co., S-14825) and a gas bubbling stone (commercial aquarium type). A piece of copper and a sample of Alconox cleaner were toxic, as was expected.

These tests furnished proof that the materials used in biofuel cell systems in this laboratory were not detrimental to the microorganisms being studied under this contract.

Another series of tests was run to determine the type and concentration of germicide that must be used to obtain sterility when cleaning the biofuel cell systems. The samples were prepared on sterile trypticase soy broth agar in sterile Petri dishes, and inoculated with E. coli; the duration of the test was five days. It was found that 35% by volume of ethyl alcohol or 1% by volume of Clorox (0.5% sodium hypochlorite, Clorox Co., Oakland, Calif.) are effective germicides.

If any of the necessary materials used in the experiments conducted under this contract had been found to be toxic to certain types of microorganisms, appropriate leaching, cleaning, or surface coating methods might have been used to remove them, but it was found that the usual methods of laboratory cleaning and sterilization of apparatus sufficed.

K. ELECTRODES AND ELECTRODE REACTIONS

1. Effect of Electrode Metal:

The electrodes used in the experiments described in this report were either platinized platinum foil, in the static glass H-cells, or platinized screen containing 90% platinum and 10% rhodium, in the plastic, continuous flow systems. Other more economically attractive electrode materials were not studied, because the emphasis was placed on optimizing the reactions.

Some limited studies of polarization and overpotentials, limiting current densities, and other phenomena of electrode kinetics were conducted, commensurate with the investigations of the co-contractors mentioned in the Introduction to this report (Section II). These studies are described in appropriate sections of this report.

Reference electrodes (saturated calomel) and Luggin capillary salt bridges were used in all experimental systems after the first month; values of electromotive potentials were based upon the reference electrodes after that time.

It was found that the anodic open-circuit potential of platinum foil in the nonflow system was approximately 0.1 volt higher than that of the platinized Pt-Rh screen, even though the fuel-anolyte in the two systems was obtained from the same mixture.

An experiment was conducted to determine whether this difference in the anodic open-circuit potentials was caused by the difference in electrode materials (i.e., platinum foil compared to platinum-rhodium screen). In this experiment, the electrodes were platinized platinum foil in both the flow and nonflow systems, and fuel-anolytes of both cells were taken from the same mixture. Again, the open-circuit anodic potential was greater in the non-flow system after 63 hours (Experiment 61), although it was greater in the flow system during the period from 18 to 63 hours.

2. Effects of Method of Bubbling Gases into Cells and Physical State of Fuel-Anolyte:

It was postulated that another possible explanation for this difference in potentials might be found in the methods of introducing gases to the electrodes, or in the accessibility of solid feces to the electrode. An experiment was conducted to determine the magnitude of these effects.

In this experiment, four cells were assembled and run simultaneously. One was a plastic cell in a flow system. The other three were H-cells (nonflow) that differed in the following respects:

(a) Gas bubblers were placed below the electrodes, as customary; (b) gas bubblers were placed above the electrodes; and (c) gas bubblers were placed below the electrodes as usual, but only the supernatant liquid of a feces-urine mixture was used as the fuel-anolyte. The experimental conditions were the same as those described in Table I of the Appendix, except that foil electrodes were used in the flow system, and the catholyte contained 2-1/2 weight percent NaCl and 2-1/2 percent KCl.

The results are summarized in Table V (Experiment 65), and it is apparent that the peak anodic power density was not improved by bubbling the gases directly into the plastic cell (compare the data of Experiment 65 with Experiment 1). However, it was found that the open-circuit anodic potential of the flow system exceeded that of the normal H-cell for a period of time (see Figure 12). The reason for the delay in attaining an open-circuit potential of approximately 500 millivolts in the plastic cell, as well as the reason for the potential of the normal H-cell ultimately exceeding that of the plastic cell, have not been determined but are doubtless due to microbiological reactions.

The data of Experiment 65 further showed that placing the gas bubblers below the electrodes, as has been done in most of the experiments in this investigation, was more effective for agitation but did not cause any higher cathodic potential than if the bubbler were above the electrode so that it merely saturated the electrolyte but did not bubble gases across the electrode. The data also showed that the supernatant fuel-anolyte mixture containing no solids was essentially as effective as the fuel-anolyte containing solids.

3. Determination of Whether the Anode or Cathode Reaction is Limiting

In most of the experiments under this contract, the anode and cathode areas were equal for simplicity. However, it was of interest to know which reaction (anodic or cathodic) was rate-limiting. Therefore, an experiment was conducted in which two flow cells were used (in parallel), both having electrodes of unequal areas, to determine which reaction is rate limiting. The electrodes are described below:

Cell I - Anode: Platinized Pt foil, 1 sq. in.; non-opposing faces coated with water repellent paint

Cell I - Cathode: Platinized Pt foil, 2-1/8 in. clear dia., 3.56 sq. in.

Cell II - Anode: Platinized Pt foil, 2-1/8 in. clear dia., 3.56 sq. in.

Cell II - Cathode: Platinized Pt foil, 1 sq. in.; non-opposing faces coated with water repellent paint

In all other respects, the experimental conditions were the same as those described in Table I of the Appendix. A summary of the results is presented in Table V (Experiment 64).

On the basis of these data, it is apparent that the cathode reaction is limiting, since the use of a larger cathode permits more current to flow.

Further experiments were conducted, in an effort to determine the extent of limitation of the cathode reaction. The experimental conditions were essentially the same as those described in Table I, except that the anode area was varied; cathode-to-anode area ratios of 2:1, 4:1, and 8:1 were employed.

The results of the experiments (Experiments 68 and 75) are summarized in Table V. On the basis of these experiments, it appeared that the limiting electrode area ratio was approximately 8 cathode: 1 anode.

L. SEPARATION OF CHEMICAL-ELECTROCHEMICAL FROM BIOCHEMICAL FUEL CELL REACTIONS

A series of experiments was conducted to separate the chemical and biochemical reactions in a biofuel cell. The experimental three-arm H-cell employed in these experiments is shown in Figure 13. The conditions of the experiment (electrodes, O-rings, catholyte, and waterproof paint) were the same as those shown in Table I, except for using the three-arm H-cell instead of the conventional two-arm H-cell (see Figure 2). In this application, the center arm was the sterile anode chamber, one of the outer arms was the cathode chamber, and the other outer arm was the biological (non-sterile), electrodeless chamber. The procedure was modified by sterilizing a portion of fuel-anolyte (by autoclaving) and putting it into the anode chamber; in this chamber only electrochemical reactions could occur. A portion of nonsterile fuel-anolyte was placed in the biological, electrodeless chamber, where only biochemical reactions could occur.

The anode chamber was separated from both the cathode and biochemical chambers by inserting cellulose acetate membranes at the O-ring joints.

A conventional two-arm H-cell was used as a control in this experiment. The conditions for this cell again were the same as those listed in Table I, except that the fuel-anolyte was a portion of the sterilized fuel-anolyte prepared for the anode chamber of the three-arm cell. In this manner, any strictly chemical-electrochemical reactions occurring in the fuel-anolyte of the anode chamber of the three-arm cell would be duplicated in the two-arm cell, and the effects could be separated from those of other types of reactions occurring in the three-arm cell. The other type of reaction occurring in the three-arm cell would involve the transport of electrochemically active molecules formed in the biological chamber, through the cellulose acetate membrane, to the anode chamber. Therefore, this experiment showed whether electrochemically active products were formed as a result of metabolism of the indigenous microorganisms in human waste.

The results of the experiment are summarized in Table V (Experiment 44). The cells are identified as follows:

I = Two-arm Cell; II = Three-arm Cell

The anodic open-circuit potential of the three-arm cell seemed to be slightly better than that of the two-arm cell during the first 76 hours of the experiment, but during the next 60 hours there was no definite difference in the potentials of the two cells. The attainment of only slight differences of potentials was considered to be due possibly to limited diffusion through the cellulose acetate membrane.

The experiment was repeated, but the separator between the biological outer chamber and the anode inner chamber was a 0.45 micron Millipore filter paper, instead of the cellulose acetate used previously. The Millipore filter paper was chosen because its pore size was sufficiently small to prevent diffusion of microorganisms to the anode chamber, but large enough to permit rather free diffusion of any electrochemically active molecules formed in the biological chamber.

Again, the anodic open-circuit potential of the three-arm cell was slightly better than that of the two-arm cell, by perhaps 0.1 volt, for the period from 17 hours to 75 hours, even though the open-circuit potential of the three-arm cell was originally approximately 0.225 volt less than that of the two-arm cell (the two cells should have been almost duplicates at the beginning of the experiment). Over the period from 85 hours to 140 hours, the open-circuit potentials of the two cells did not differ significantly.

Two polarization tests were made with the three-chamber H-cell, and the results are presented in Table V (Experiment 46). The polarization tests were reasonably separated in time, and it appears that electrochemically active compounds were formed by metabolic activity, and that these compounds then diffused to the anode and caused an increase in anodic power density over that obtained initially through purely chemical reactions.

The experimental method was modified by placing a second anode in the biological chamber of the three-arm H-cell; in all other respects, the experiment was the same as that described above. The purpose of this experiment was to determine the difference in electrochemical behavior between a biological and a nonbiological anode, with provision for interdiffusion. It was found that the open-circuit anodic potential obtained from the nonsterile (outer) anode was greater than that obtained from the sterile anode for the first 50 hours. After that time, the open-circuit potential obtained from the sterile anode was approximately 200 millivolts greater than that obtained from the nonsterile anode (referenced to saturated calomel).

The data indicate that although the nonsterile system produced a better potential originally, the chemicals present in that system apparently became depleted rather rapidly, while those of the sterile system did not. There was a question regarding whether the human waste that was autoclaved was completely sterilized, because the variation of anodic open-circuit potential at the "sterile" anode was generally similar to that of the nonsterile anode, except for a delay in attaining the greatest potential. The data obtained were significant, however, in showing the effects of metabolic activity on potential.

No polarization or power data were obtained from this experiment, because the longer electrolytic path between the cathode and biological anode, the presence of the nonbiological anode between the cathode and the biological anode, and the addition of the Millipore filter between the biological and nonbiological anodes, increased the internal resistance of the cathode-biological anode circuit above that of the cathode-nonbiological anode circuit. See Table V, Appendix A, Experiment 11.

M. SEPARATION OF CHEMICAL-BIOCHEMICAL FROM ELECTROCHEMICAL FUEL CELL REACTIONS

Some experiments were conducted to determine the extent of electrochemical reactions in the biofuel cell, which occur in the proximity of the electrode, in contrast to purely chemical-biochemical reactions, which are independent of the electrodes.

The method employed was to run flow and non-flow systems simultaneously under the conditions described in Table I, and compare the variation in electrochemical properties with time. In the nonflow system, the total volume of fuel-anolyte mixture (approximately 80 milliliters) was confined near the electrode (see Figure 2), whereas in the flow system only a small portion of the fuel-anolyte was actually in the fuel cell at a given time and the remainder was in the reservoir (see Figure 4). The ratio of total time:cell time provided an indication of the ratio of the amount of time the fuel-anolyte was in the fuel cell and could contact the electrode for electrochemical reactions, to the total amount of time the mixture was in the system. For diffusion, it was recognized that the distance of fuel-anolyte from the electrode was greater in the nonflow system than in the flow system (considering only fuel-anolyte mixture actually in the biofuel cell); there was a maximum of approximately 2 inches in the former and 1/2 inch in the latter. However, because of the agitation provided in the nonflow system by bubbling helium into the mixture, the diffusion rates were probably high enough that they were not the limiting steps in the reaction. The ratio of total time:cell time was assumed, therefore, to be 1.0 in the nonflow system, and varied with the ratio of total volume of fuel-anolyte in the flow system to the volume of fuel-anolyte contained in the fuel cell (approximately 31 milliliters).

In the first experiment, the ratio of total volume:cell volume was 9.0. No current was drawn during this experiment, except for the minor amount withdrawn by the voltmeter (10^6 ohms input impedance) for 3 seconds each half-hour.

The purpose of that experiment, the first in the series, was to establish reproducibility of the two systems. It was noted that the open-circuit potential of the nonflow system employing a platinized platinum foil anode was approximately 100 millivolts greater than that of the flow system employing a platinized screen of 90% platinum - 10% rhodium. (This had been noted, and is discussed in further detail in Section III.K.1, Effect of Electrode Metal.)

In a second experiment, the ratio of the total volume of anolyte in the flow system to the volume of anolyte in the biofuel cell was 12.2. Experimental conditions were the same as those described above, except that the current was maintained constant at a given time by means of the galvanostat, and varied during the experiment from 20 to 34 microamperes. The flow and nonflow cells were connected in series to the galvanostat, so that the same current was drawn from both cells. The current densities

were in the range from 2.88 to 4.89 and from 0.81 to 1.38 milliamperes per square foot, for the nonflow and flow systems, respectively.

The data were plotted for the flow system on both a cell time and total time basis. There was no direct relationship between the anodic open-circuit potentials of the two systems as a function of time, on either basis.

Further work was designed to relate the ratio of electrode surface:fuel-anolyte volume to the time.

In a slightly different approach toward separating chemical-biochemical reactions from electrochemical reactions, two nonflow cells were assembled under the conditions described in Table I. Current was withdrawn from one cell, maintained at a constant rate by means of the galvanostat, while the other cell was a control from which only infinitesimal amounts of current were withdrawn; i.e., only the small current that flowed through the voltmeter (10^6 ohm input impedance) during the 3-second voltage reading each half-hour during the test. The current withdrawn from the cell varied from 20 to 62 microamperes (2.88 to 8.94 milliamperes per square foot) and a total of 47.5 coulombs were withdrawn during the experiment.

The data are presented in Table V (see Experiment 55), and indicate that the withdrawal of the current caused the electrical power output to be decreased significantly, from a peak anodic power density of 1.75 milliwatts per square foot of electrode surface after 30 coulombs of electricity had been withdrawn from the cell (total elapsed time 146 hours), to 0.2 milliwatts per square foot after 47.6 coulombs had been withdrawn (total elapsed time 339 hours). This decrease in power was not caused by time alone, since the control cell (essentially no current withdrawn) gave a peak anodic power density of 2.1 milliwatts per square foot after 382 hours total elapsed time. The withdrawal of 47.6 coulombs of electricity did not represent the maximum that could be obtained, but the experiment was terminated when it seemed to be impractical to continue because of the low power level obtainable at that time.

In a final experiment in this series, the flow and nonflow systems were used in the same manner as described above, except that the ratio of electrode area to fuel-anolyte volume was the same in both systems, as shown in the following tabulation:

System	Electrode Area (sq. in.)	Fuel-Anolyte Volume (ml.)	Ratio of Electrode Area to Fuel-Anolyte Volume
Flow	3.54	300	0.0118
Nonflow	1.00	85	0.0118

N. REPRODUCIBILITY; STATISTICAL ANALYSIS

A limited number of reproducibility tests were conducted in H-cells under the conditions described in Table I, and it was found early in this program that the open-circuit potential varied as much as 70 percent and the short-circuit current 700 percent. In the continuous flow system reproducibility tests using plastic cells, two cells were connected in series, the anolyte and catholyte reservoirs were common to both cells, and everything else was duplicated in both cells (electrodes, structural material, etc.), except the membranes. The open-circuit potentials in two flow cells varied as much as 95 percent (55 percent at the time of starting the test).

These early data indicated that a higher degree of reproducibility was necessary in these studies, and it was obtained by standardizing and improving the techniques used.

The reproducibility of data was established by making reproducibility tests every three or four runs. These tests were not time-consuming and lasted approximately 24 hours. They provided valuable information in verifying that the system was the same and invariant with time, and in eliminating questions regarding the effects of possible contamination and of changes in sources of materials or in techniques that varied over an extended period of time.

The reproducibility tests were made in several phases, to determine whether chemical and bacteriological complexities affected the reproducibility. The phases are described below:

Phase I	Escherichia coli in brain heart infusion
Phase II	Feces-urine supernatant, E. coli, and brain heart infusion
Phase III	Feces-urine supernatant, with less E. coli and brain heart infusion than in Phase II
Phase IV	Feces-urine mixture with only indigenous microorganisms
Phase V	Feces-urine mixture with centrifuged E. coli

In Phase I, the fuel-anolyte was as simple, and hopefully as invariant, as possible. This system employed a single microorganism (fresh Escherichia coli) in a pure nutrient (brain heart infusion). The E. coli culture was added to sterile brain heart infusion media to provide a mixture containing 10% by volume of E. coli culture and 90% media. The E. coli was grown in brain heart media. Four cells were assembled, the test covered a period of approximately 65 hours, and the reproducibility was acceptable, as shown by the data in Table X. The data in Table X reflect the worst conditions; i.e., the maximum differences in open circuit potentials of the four cells at any given time.

Phase II demonstrated the reproducibility of a fuel-anolyte similar to that of Phase I but containing the supernatant liquid of a feces-urine mixture. The original feces-urine mixture was prepared as described in Table I. This mixture was centrifuged at 5000 rpm for 20 minutes at 15°C. A portion of the supernatant liquid (50 milliliters) was mixed with 40 milliliters sterile brain heart infusion and 10 milliliters fresh E. coli culture (also in brain heart infusion). Again, four cells were prepared, the test lasted approximately 16 hours, and the reproducibility was satisfactory, as shown by the data in Table X.

Phase III was next, and the fuel-anolyte was essentially the same as in Phase II but with a lower concentration of E. coli and brain heart infusion media. In this phase, ½ milliliter of E. coli culture was grown in 500 milliliters brain heart infusion, and 10 milliliters of this inoculum was added to 90 milliliters of the human waste supernatant. Again, four essentially identical cells were prepared, the test lasted approximately 80 hours, and the reproducibility was satisfactory.

In Phase IV, the feces-urine mixture was employed as the fuel-anolyte, with no additions of microorganisms or nutrient medium. The mixture was the same as that described in Table I. This test lasted approximately 18 hours, and the reproducibility of the four cells assembled was satisfactory. The reproducibility of this system is shown in Figure 14 and in Table X.

In Phase V, the reproducibility was determined of a fuel-anolyte to which an external microorganism was added, but essentially without accompanying nutrient. An E. coli suspension in brain-heart infusion was centrifuged at 5000 rpm for 20 minutes; the supernatant liquid was removed, and the E. coli was suspended in 1 milliliter sterile, deionized water. The resuspended E. coli (1 milliliter) was added to the feces-urine mixture, which was prepared as described in Table I. Four similar cells were prepared, the test lasted approximately 50 hours, and the results were satisfactory. The reproducibility became poorer with time, and one of the cells appeared to introduce broader variations than the other three cells. These variations are shown in Table X.

The pH of all cells was 8.7 initially, and 8.4 - 8.6 at the completion of the test. The purpose of the tabulation showing the variation of the statistical data with time is to illustrate the effect of metabolic reactions on the variability of anodic open-circuit potentials. The reproducibility of the open-circuit potentials early in the Phase V (i.e., the first 24 hours) were rather good, especially for three of the cells, and the variance was only 12.6. However, later in the experiment, after metabolic reactions had become significant, the variability increased so that the variance over a 50-hour period reached 423. These data illustrate that even when test cells are

assembled with such techniques that they initially are reasonably identical, there is a considerable amount of variability in the potentials obtained that is due to metabolic activity, and this type of variability is much more difficult to control than the types of variations due to other causes (e.g., materials used, cleanliness of apparatus, composition and homogenization of fuels, electrode contamination, etc.)

Statistical data from typical reproducibility experiments are presented in Table XI. The data obtained during the reproducibility studies show that a satisfactory level of reproducibility was attained with the flow system in Experiments 41 and 42. The data obtained in Experiments 39 and 40, with the flow and nonflow systems, respectively, were not satisfactory; the reason was not determined, and this fact re-emphasizes the need for reproducibility experiments.

Polarization and power data were obtained with the flow system during one of the standard reproducibility experiments (Experiment 42), and the data obtained are summarized in Table V. A general description of the method employed in obtaining polarization and power data is presented in Section III.F.

The reproducibility of the nonflow system was also determined periodically during this program. The experimental conditions are described below:

Nonflow System

Cell: Glass, H-shape, O-ring type

Electrodes: Platinized platinum foil, 1 sq. in. area (non-opposing faces coated with waterproof and chemically resistant paint).

Separator, O-Rings, Catholyte, Fuel-Anolyte, and Waterproof and Chemically Resistant Paint: Same as for flowing system.

The reproducibility of these test cells reached a high degree of acceptability, so that in Experiment 62 (three nonflow test cells were used) the mean of the maximum differences of open circuit anodic potentials was 38 millivolts, the mean deviation 36 millivolts, and the variance 3. In Experiment 72, two flow-type test cells were assembled in parallel, and the statistical data covering the entire 124 hours and the final 64 hours of the experiment are summarized below.

	Entire 124 Hours	Final 64 Hours
Mean of Maximum Difference of Potentials (millivolt)	64.1	28.6
Standard Deviation	46.7	35.5
Variance	2190	1275

In addition to the anodic open-circuit potentials usually obtained in these reproducibility runs, polarization runs were also made occasionally. A summary of the data obtained from one experiment (Experiment 62) is presented in Table V of the Appendix. The three runs were made over a period of 30 hours, so there might be some minor variations in power output due to metabolic activity. However, the data showed that the open-circuit potential remained essentially the same throughout this time period. The results of a similar experiment (Experiment 56) are presented in Table V.

O. CONTINUATION OF LITERATURE SURVEYS

Throughout this program, the literature was consulted for electrochemical, microbiological, and material effects, and other pertinent information and data. These surveys were made concurrently with experimentation. A bibliography is appended.

One item of interest during this program was the proximate analysis of feces. Fortunately, this information was found in the literature, and is included in this report as Table II of the Appendix. It will be noted that only approximately 84% of the contents of feces has been identified.

P. BOMB CALORIMETRIC STUDIES

The oxygen bomb calorimeter was used to determine the relative heating values of the human waste (i.e., in the form of lyophilized feces) after various types of degradation or extent of current withdrawal. This information afforded a measure of (a) the extent of degradation of the waste, or (b) the relative amount of electrochemical energy obtainable from the waste under various conditions.

Typical data from two experiments are shown in Table XII. It may be noted that the values reported in that table (4591 and 4697 calories per gram) are higher than some values that have been calculated and reported in the literature, based upon estimated compositions of human feces and the heats of combustion of the components.

Q. MISCELLANEOUS STUDIES

In a single experiment to determine the amount of time required to possibly coat or poison the electrodes in the biofuel cells, it was found that a clean pair of electrodes functioned about the same as a pair that had been in a cell for twelve days. The decrease in electrical output with time that had been experienced was therefore not caused by continued coating or poisoning of the electrodes.

A test was made in an H-cell to determine the effect of electrode area on current density. The electrolyte was saturated KCl. At very low current densities (below 6×10^{-4} amperes per square foot, based on one side), there was no difference in the current densities, whether the electrode had only one side or both sides exposed. Above that current density, two sides conducted better than one side, up to a current density of 8×10^{-3} amperes per square foot. At still higher current densities, an electrode with only one side exposed conducted better than one with both sides exposed, possibly because of conflicting electrolytic current paths.

An experiment was conducted to compare the effects of bubbling the fuel-anolyte mixture with inert gas (helium) to provide agitation and remove gaseous products which may be either inhibitory or enhancing to biofuel cell reactions, in contrast to agitation by mechanically shaking the media.

The conditions of the experiment were essentially the same as those described for the nonflow system in Table I of the Appendix, except that two cells were assembled. The fuel-anolyte of one cell was bubbled with helium, as was customary, while that of the second cell was not bubbled with gas. Agitation was provided for the second cell by means of a mechanical shaker; the second cell was maintained gas tight to avoid loss of gaseous products. Data were obtained at various times during the experiment, and a summary is presented in Table V of the Appendix (Experiment 59).

It was apparent that bubbling the fuel-anolyte was advantageous, in contrast to mechanical agitation, because both the best anodic open-circuit potential and the short-circuit current density were better for the bubbled system. This indicated that some inhibitory substances were being removed from the cell by bubbling, and that the agitation provided by bubbling was sufficient to minimize polarization.

R. MICROBIOLOGICAL ASPECTS

1. General Considerations

The developmental aspects of biochemical fuel cell work require that only a limited microbiological research program be conducted. Indigenous organisms must be accepted, since there is no practical means at present of sterilizing human waste in space travel.

The indigenous organisms will grow according to their biochemical capabilities and under the given nutrient and physical conditions of the fuel cell. The complex nature of the metabolic reactions can be recognized in view of the number of different organisms which might grow, the variation in numbers of any type of organisms which may be eliminated in human waste, the changes associated with different diets, human physiological variance in intestinal digestion of food, and microbiological variations such as mutation and synergistic and antagonistic reactions between microbes.

The supply of human feces used in most of the experiments described in this report was obtained by combining the fecal output of several apparently healthy individuals on a special low-cellulose diet. The feces was then frozen and stored for convenience. The electrochemical activity and power output of these mixtures has been measured, primarily at room temperature and without additives. Thus, the electrochemical activity is related to indigenous microbial metabolism.

Theoretically, the most desirable system would be one stabilized by the presence of one or a few types of microorganisms in large numbers and yielding satisfactory electrochemical activity. Since many of the microbes present in feces at the time of elimination by the human will not grow at room temperature or thrive without nutrients or changes in conditions, the microbial flora will change significantly as the feces decompose under indigenous attack. This microbial change may consume considerable time without significant electrochemical advantage. Thus, the desirable system should have large numbers of electroactive microbes to enhance power output. The described situation may be accomplished by (1) inoculating useful microbes into feces at the start of decomposition, or (2) inoculating relatively small quantities of fresh feces into relatively large quantities of actively decomposing feces, as in typical sewage treatment.

2. Transfer (Preculturing) Methods

A single experiment was conducted in an attempt to maintain electrochemical activity of the human waste at a peak level, by transferring a portion of the waste at its peak power output (e.g., 24 hours old) to fresh waste; the fresh waste constituted 90 percent of the

mixture by weight. The fresh material contained 30 grams of feces and 100 milliliters of urine (both non-sterile). The original attempt was neither harmful nor beneficial, and the mixture of old and new material behaved essentially the same as new material.

Organisms from the indigenous mixture in feces and urine were isolated by adding 3% agar (in a 1.5% salt solution) to an equal volume of urine-feces and incubating plates anaerobically and aerobically. A number of organisms grew moderately well on the surface of agar as isolated colonies. Colonies of the microbes were transferred to nutrient broths, and these growths were then plated on trypticase soy agar. Isolated colonies, examined microscopically for purity, were transferred to sterilized urine agar and urine-feces agar plates to affirm growth on these nutrients. Thus, the purity of the cultures initially isolated was established.

Several Gram positive rods (Bacillus species by morphology and culture), several Gram negative rods, and one Gram positive coccus were isolated. Precise microbiological identification was not justified, since more of the organisms showed significant electrochemical activity in the fuel cell. One of the above Gram positive rods, which does not obligatively require urea, displayed a vigorous growth rate.

Some of the more rapidly growing microorganisms indigenous to human waste were later isolated from the urine-feces mixture described in Table I. Four nonflow cells were assembled: One was a control cell, assembled exactly as described in Table I; the second cell contained a portion of a strain of bacteria (identified as culture #2) isolated from human waste on agar; the third contained another strain (culture L) isolated from the waste in similar fashion; and the fourth contained a strain (No. 11) which had been obtained as a contaminant from an H-cell that had been given a routine test for sterility, after the usual cleaning process following use of the H-cell as a biofuel cell. The contaminant strain (No. 11) grew profusely in trypticase soy agar medium, and was considered to be a logical choice for metabolic and possibly electrochemical reaction in the fuel cell.

In each of the three cells described above, where strains of isolated bacteria were added to the fuel-anolyte, the addition was made to mixtures of indigenous microorganisms rather than to sterilized waste mixtures. The methods employed in growing the cultures are described in Appendix C.

It was found that the anodic open-circuit potentials of the four cells were essentially the same, within approximately 25 millivolts throughout most of the experiment. It was also noticed that the anodic open-circuit potentials of all four cells began to change

significantly after 35 hours, and went from approximately -460 millivolts to -650 millivolts (saturated calomel reference) at the end of an additional 40 hours, where they remained essentially constant for the remaining 48 hours of the experiment. These curves are shown in Figure 15 of the Appendix.

Polarization and power data obtained with strain No. 11 (i.e., the contaminant from the H-cell) gave an unusually high current density and anodic power density (see Experiment 69, Table V of the Appendix). Although the possible sources of error were checked (e.g., contaminated electrodes or gas manifolds, etc.), no error was found in the data.

The experiment was repeated for verification (see Experiment 73, Table V of the Appendix). Again, four cells (nonflow type) were assembled. One was a control cell, assembled under the conditions described in Table I; the second cell contained bacteria strain No. 2; the third sample contained a new sample of bacteria strain No. 11; and the fourth contained bacteria strain No. 11 from the previous experiment, added to fresh urine-feces mixture (10% old fuel-anolyte mixture and 90% new mixture). The repeat experiment did not verify the previous high values of potential.

It was noted, however, that the phenomenon of the agreement of the anodic open-circuit potentials with time for the four cells was verified, even during periods of rapid changes of potential. The anodic open-circuit potentials of the four cells were still nearly the same at any given time (though not quite as good as the agreement in the previous experiment), and the potentials of all four cells simultaneously began to change after approximately 35 hours of the test, changed about 175-200 millivolts over the next 15 hours, and remained essentially constant over the remaining 100 hours of the experiment.

The relatively large changes in potential doubtless were caused by metabolic reactions. These experiments showed that the rates and extent of the metabolic reactions of indigenous microorganisms were not significantly affected by the addition of selected microorganisms to the indigenous system.

3. Addition of Specific Microorganisms

Although the majority of the investigations were conducted with indigenous microorganisms, a limited amount of study was devoted to the determination of the effects caused by adding selected microorganisms to feces-urine mixtures.

In one experiment, conducted in the flow system under the conditions described in Table I, 1 percent by volume of a culture of Proteus mirabilis (grown in nutrient broth) was added to the fuel-anolyte after the potential had become stable. The reason for choosing Proteus mirabilis was that it is a prolific producer of hydrogen sulfide, and

the primary goal of this type of experimentation, using pure strains of microorganisms, was to establish biochemical-electrochemical reaction mechanisms, in an applied sense. Proteus mirabilis is aerobic, facultatively anaerobic, and is indigenous to human fecal matter.

The addition of Proteus mirabilis to non-sterile human waste after a period of stabilization of the anodic open-circuit potential (47 hours) produced no noticeable effect. This result may have been caused by the fact that the material utilizable by P. mirabilis may have been depleted by indigenous microorganisms and no longer available when the culture of P. mirabilis was added.

In another experiment, conducted as above, 1 percent by volume of a culture of Escherichia coli was added instead of the P. mirabilis. The addition of E. coli was made after the anodic open-circuit potential had become stabilized (48 hours), and again there was no noticeable effect on the anodic potential. The same explanation may apply as in the case of the P. mirabilis.

S. PRELIMINARY EVALUATIONS OF WASTE FUELS

The present investigation was based upon the evaluation of human waste as a biofuel, as specified in the contract. However, a few of the earliest experiments were designed to evaluate the effect of the addition of certain nutrients to human waste, to which microorganisms had been added in many cases; in some experiments the pH and/or the temperature was controlled. A few experiments were conducted to evaluate biocathodic microorganisms in contrast to the non-biological cathode. These experiments are described in further detail in Appendix D.

IV CONCLUSIONS

On the basis of the experiments conducted under this contract, energy is available in human waste that can be withdrawn electrochemically. Though this energy is available at relatively low power levels, it has been demonstrated that electrochemical devices of the types described in this report are feasible, particularly if other fuels are used. The technique may be extended to terrestrial use by employing other indigenous waste materials such as animal waste, garbage, plants of certain types, and others. The investigations reported herein also indicate that electrochemical energy can be used to degrade human waste.

Power densities as high as 60 milliwatts per square foot appear to be obtainable from human waste as a biofuel, since the cathode (non-biological) reaction was found to be rate-limiting, but higher power densities probably could be obtained from other biofuels such as those listed above. It was also determined that electrochemical energy could be obtained from human waste at a rate of approximately 0.032 watts per gram, but this value could probably be increased by using other fuels, such as those listed above.

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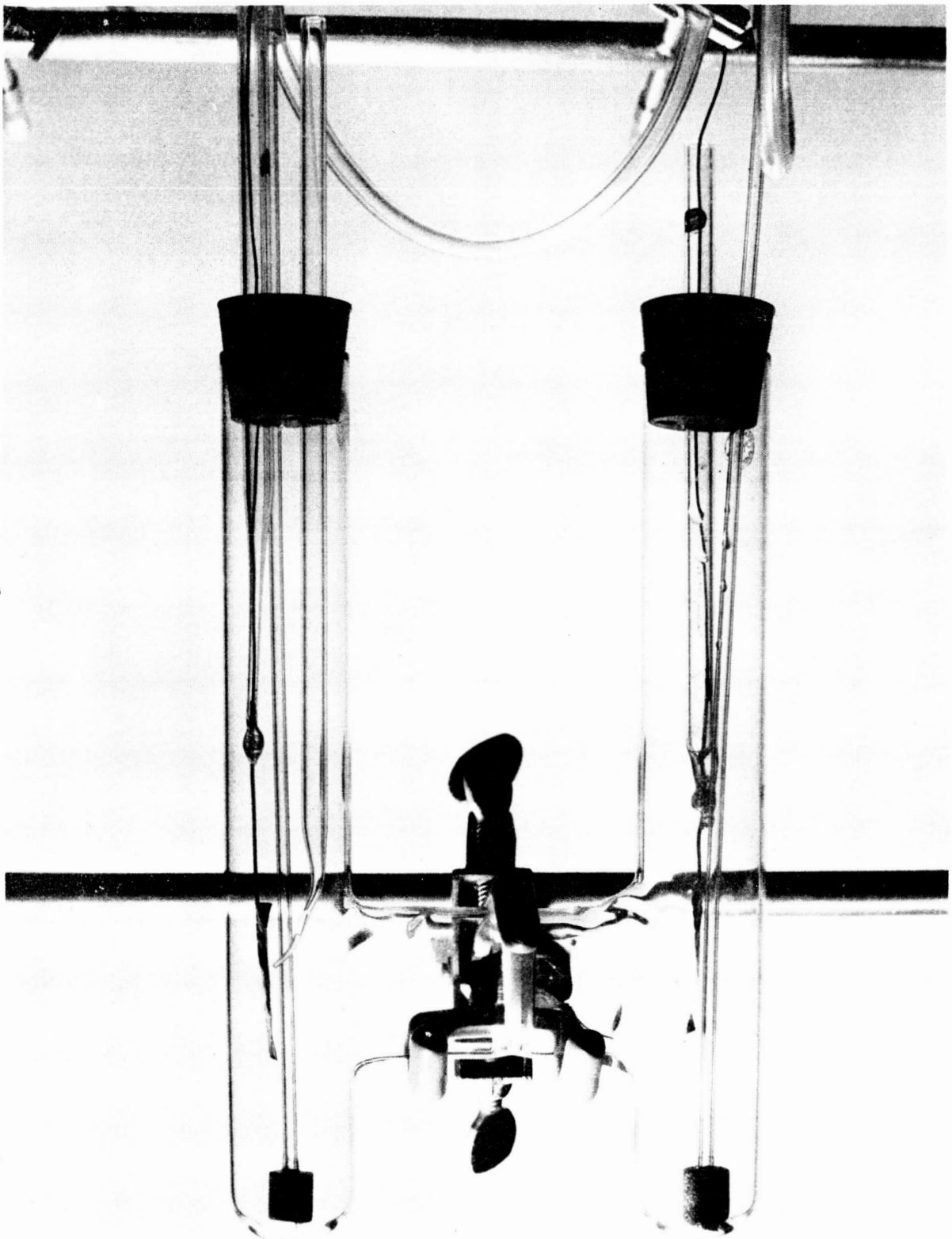
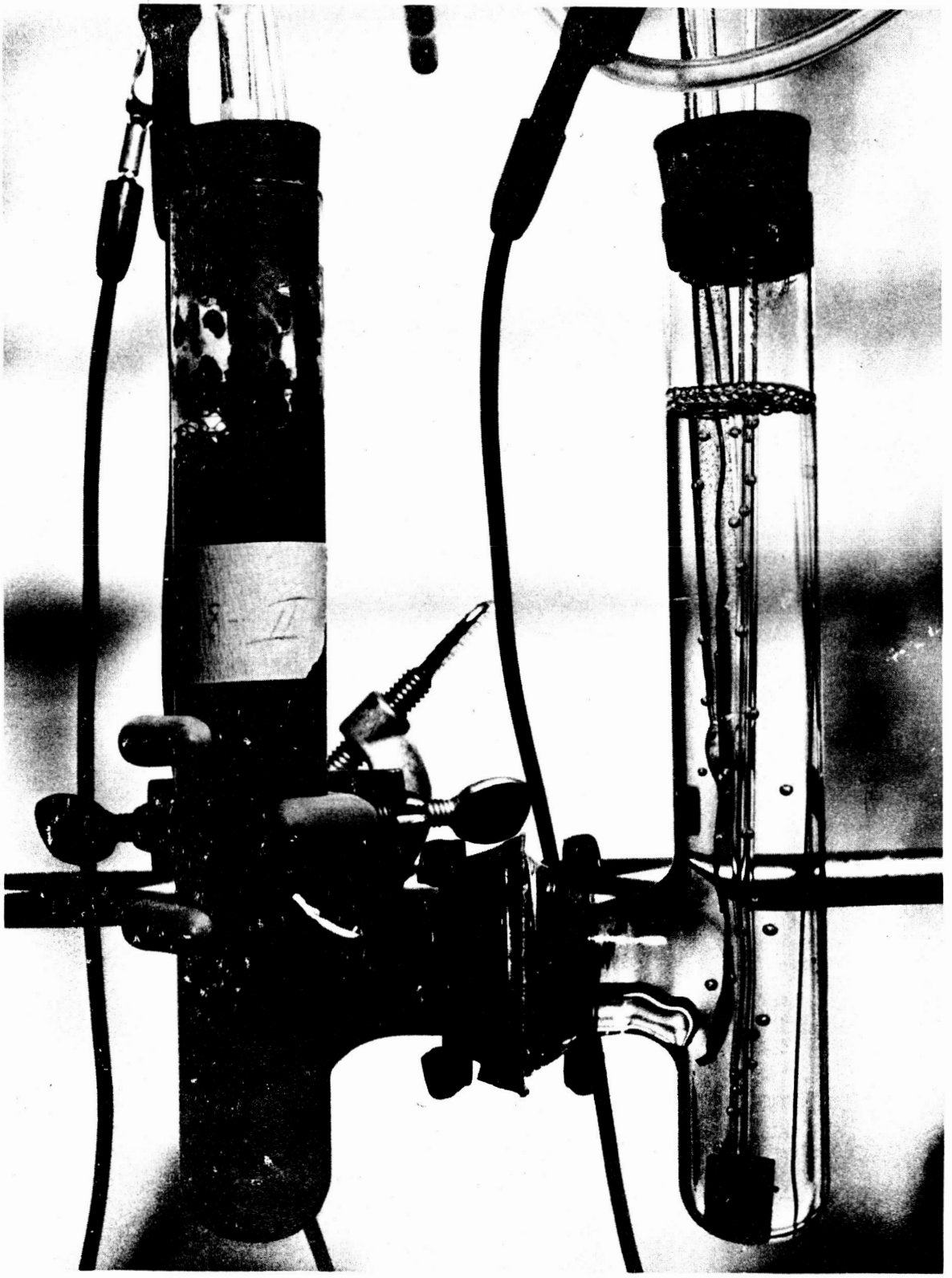
H-CELL; AGAR PLUG TYPE (CLOSEUP)



R-16,365
Neg. 4831-4

Figure 1

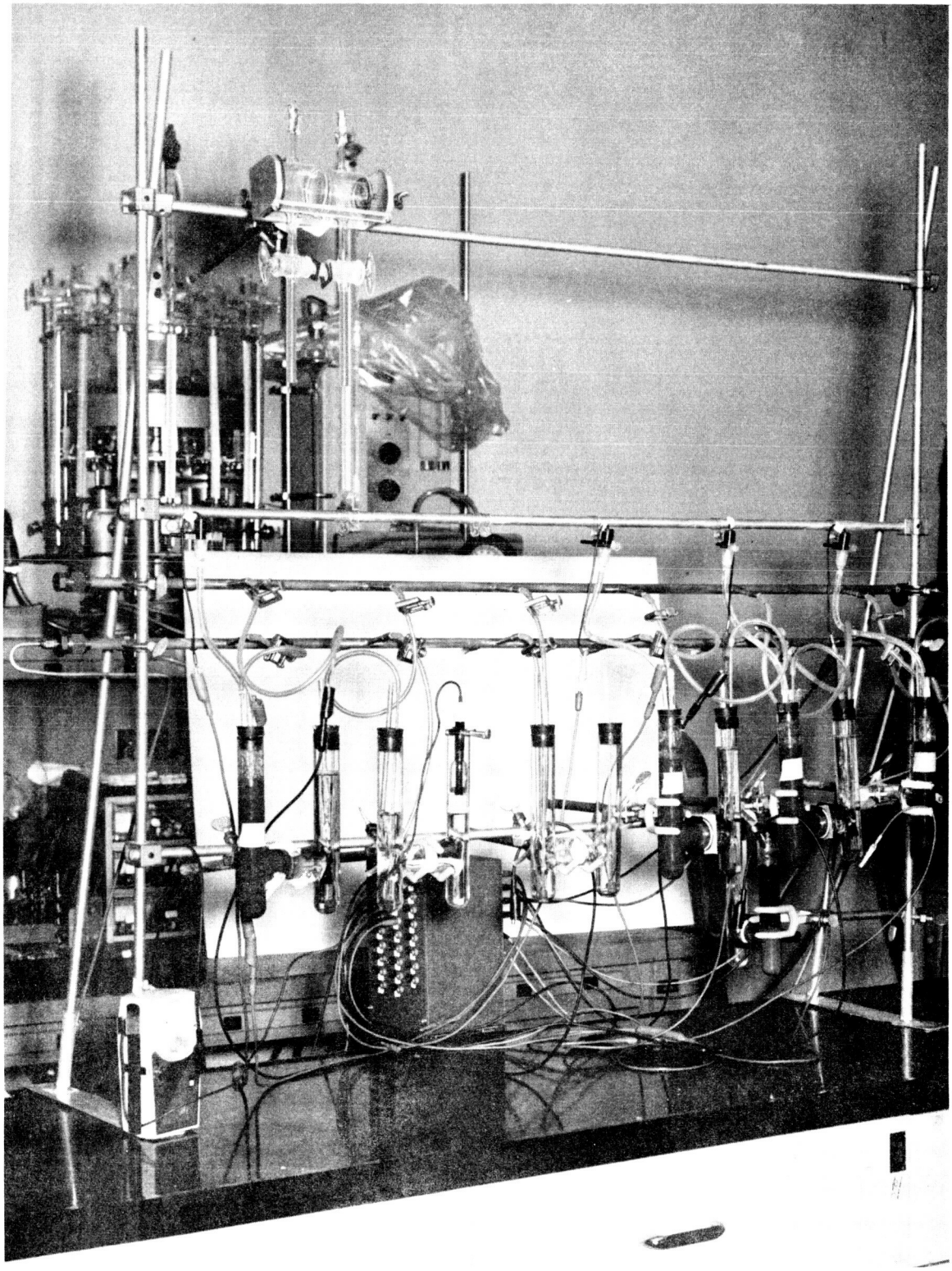
H-CELL; O-RING JOINT TYPE (CLOSEUP)



R-16,217
Neg. 4831-3

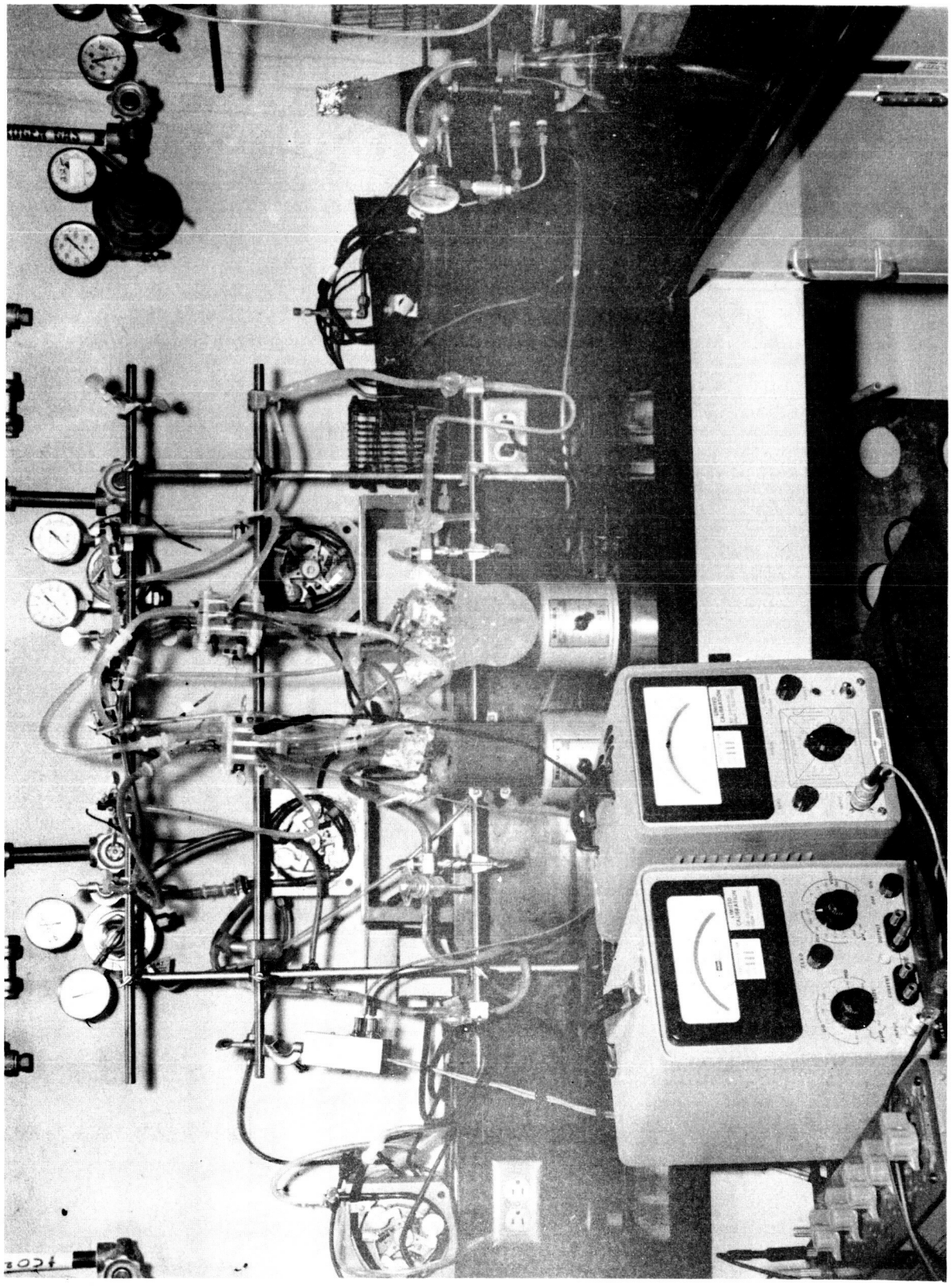
Figure 2

BIOFUEL CELL, CONTINUOUS FLOW SYSTEM



R-16,363
Neg. 4831-1

BIOFUEL CELL, CONTINUOUS FLOW SYSTEM



R-16,215
Neg. 4805-1

Figure 4

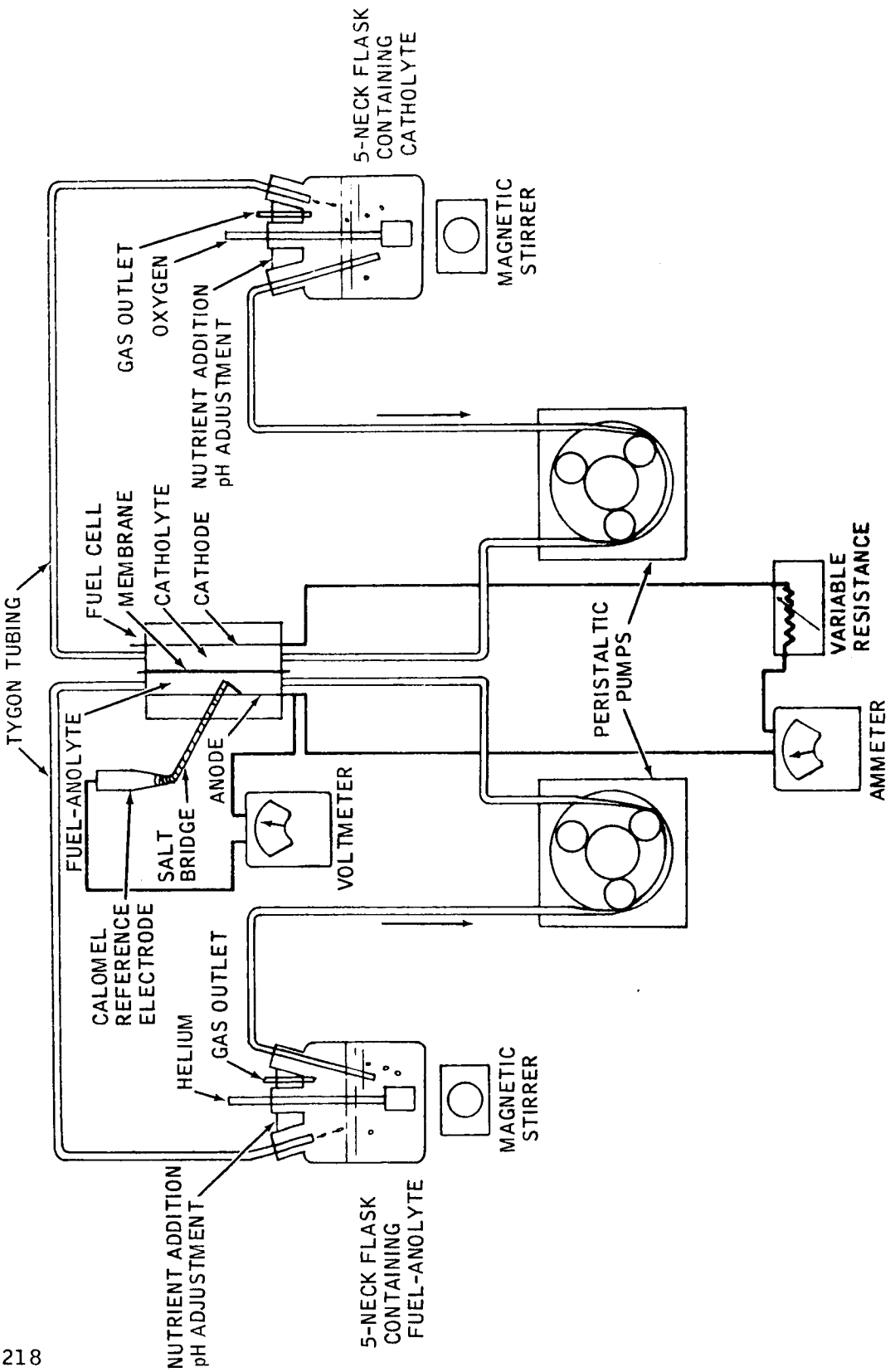
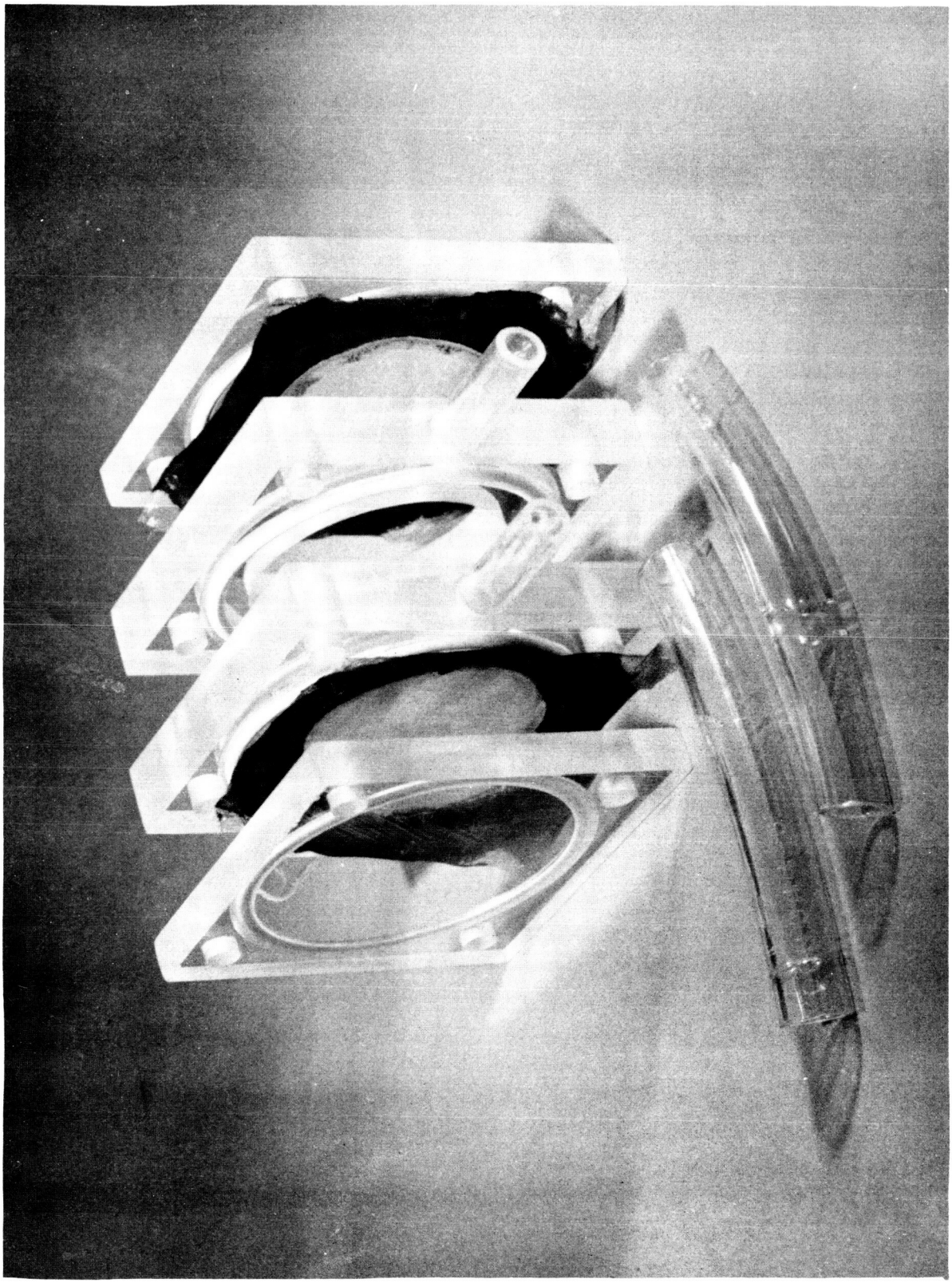

BIOFUEL CELL CONTINUOUS FLOW SYSTEM
5-NECK FLASK CONTAINING CATHOLYTE
MAGNETIC STIRRER
GAS OUTLET
OXYGEN
NUTRIENT ADDITION pH ADJUSTMENT
FUEL CELL MEMBRANE
CATHOLYTE
CATHODE
TYGON TUBING
FUEL-ANOLYTE
SALT BRIDGE
ANODE
VOLTMETER
PERISTALTIC PUMPS
VARIABLE RESISTANCE
AMMETER
CALOMEL REFERENCE ELECTRODE
HELIUM
GAS OUTLET
MAGNETIC STIRRER
NUTRIENT ADDITION pH ADJUSTMENT
5-NECK FLASK CONTAINING FUEL-ANOLYTE
R-19,218

Figure 5

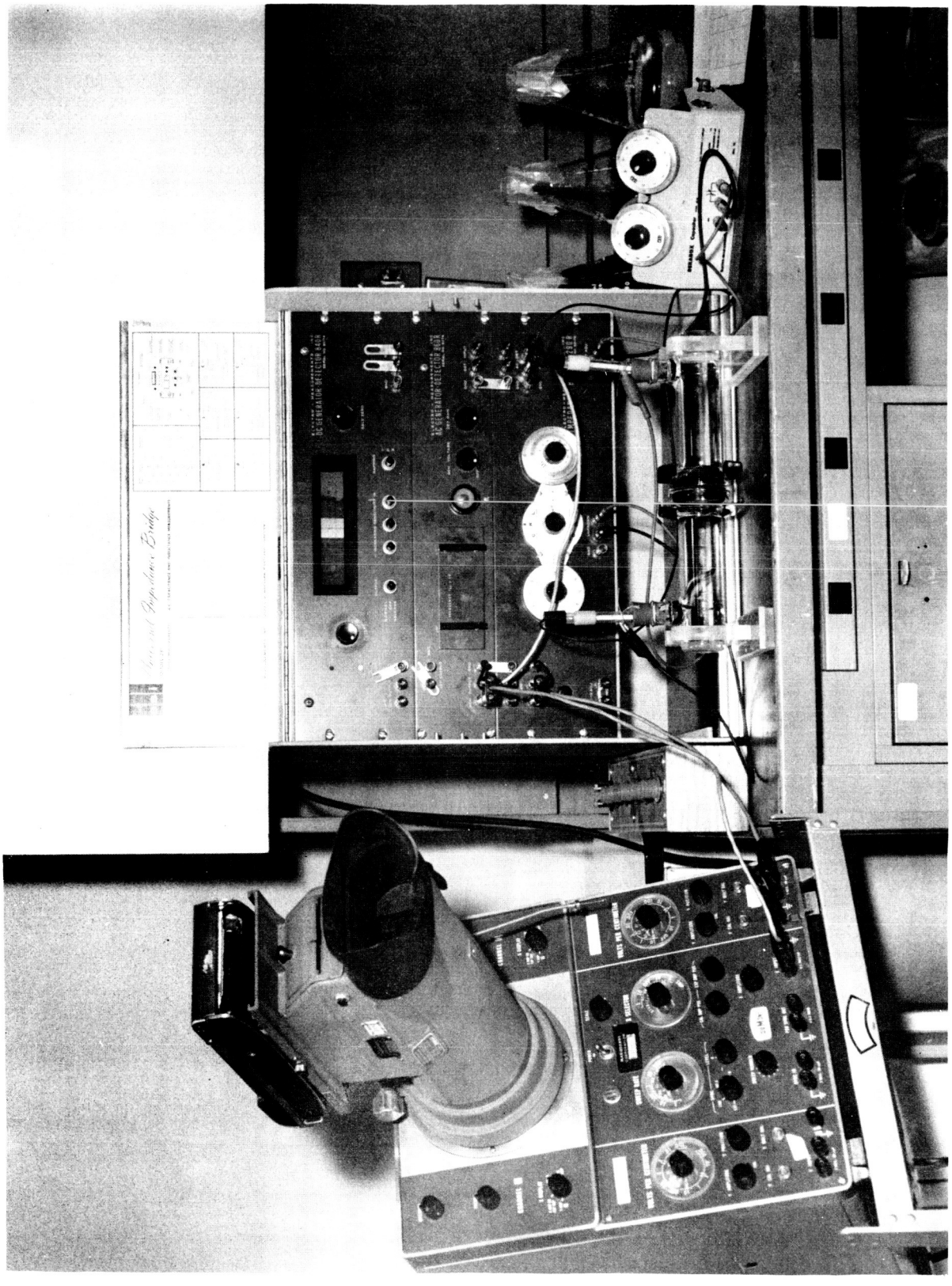
PLASTIC CELL, DISMANTLED



R-16,216
NEG. 4702-31CN

Figure 6

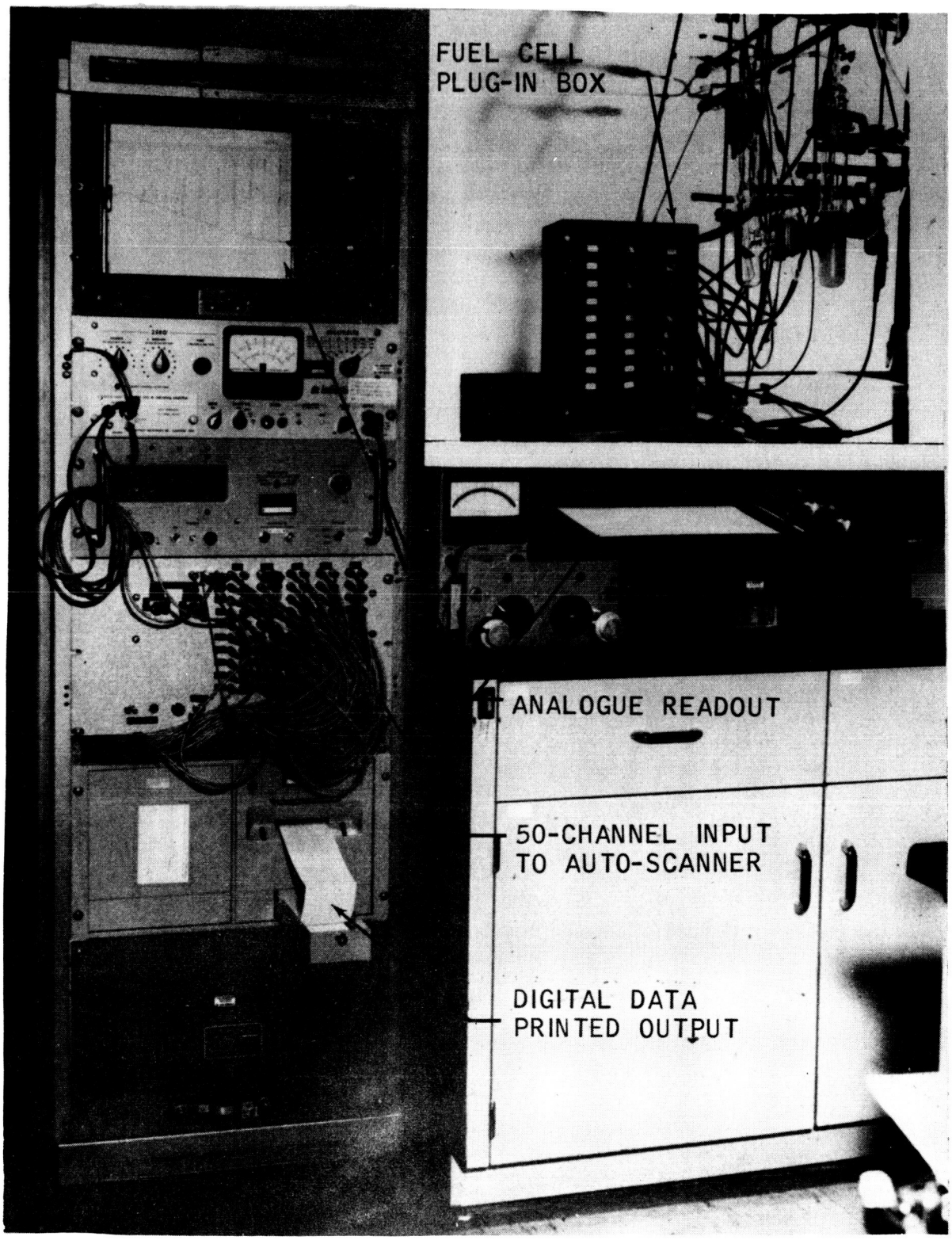
IMPEDANCE BRIDGE AND A-C CONDUCTIVITY MEASUREMENT EQUIPMENT



R-16,214
Neg. 4841-3

Figure 7

FUEL CELL DATA READOUT SYSTEM

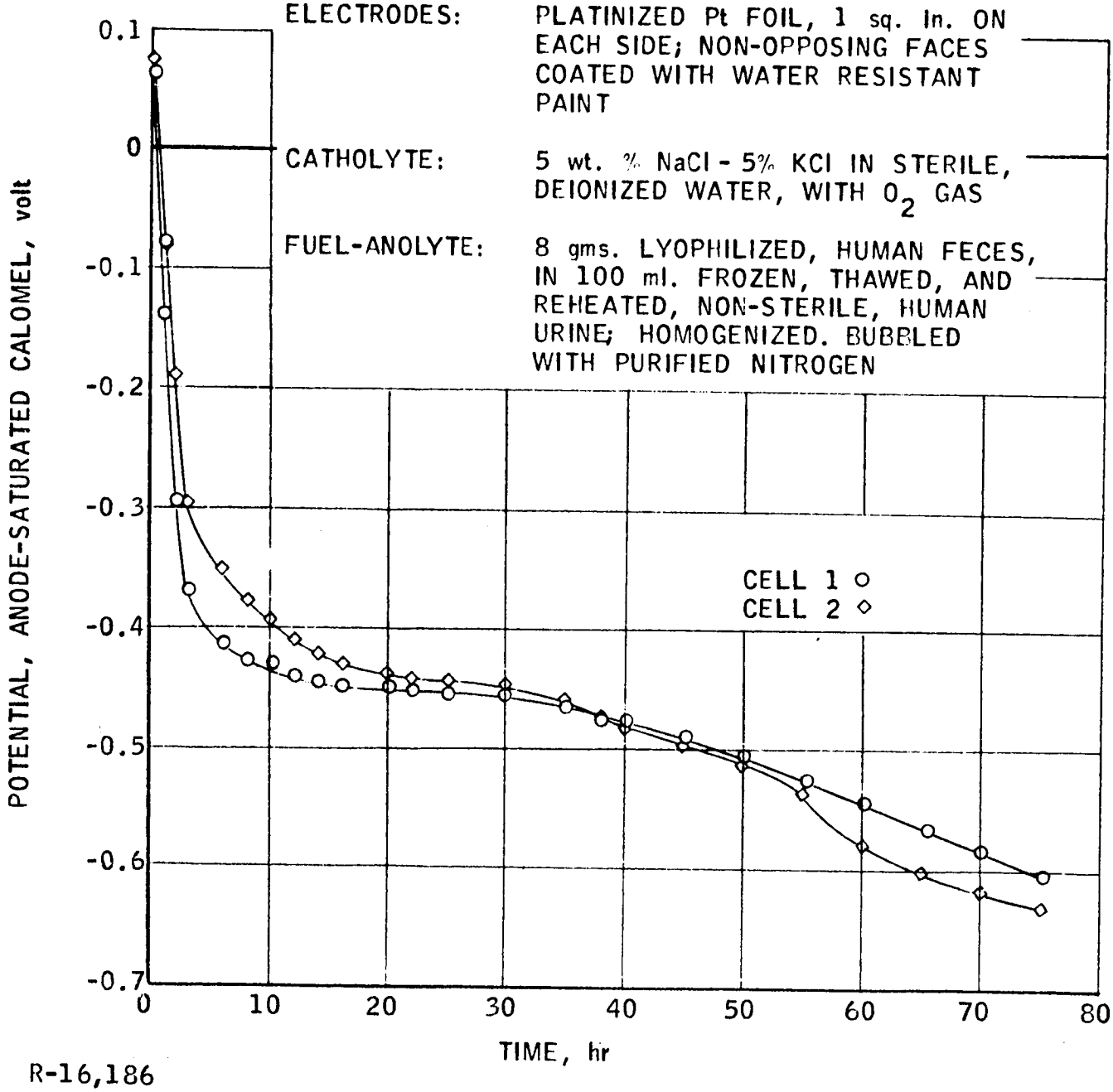


R-16,364
NEG. CAC4841-4

Figure 8

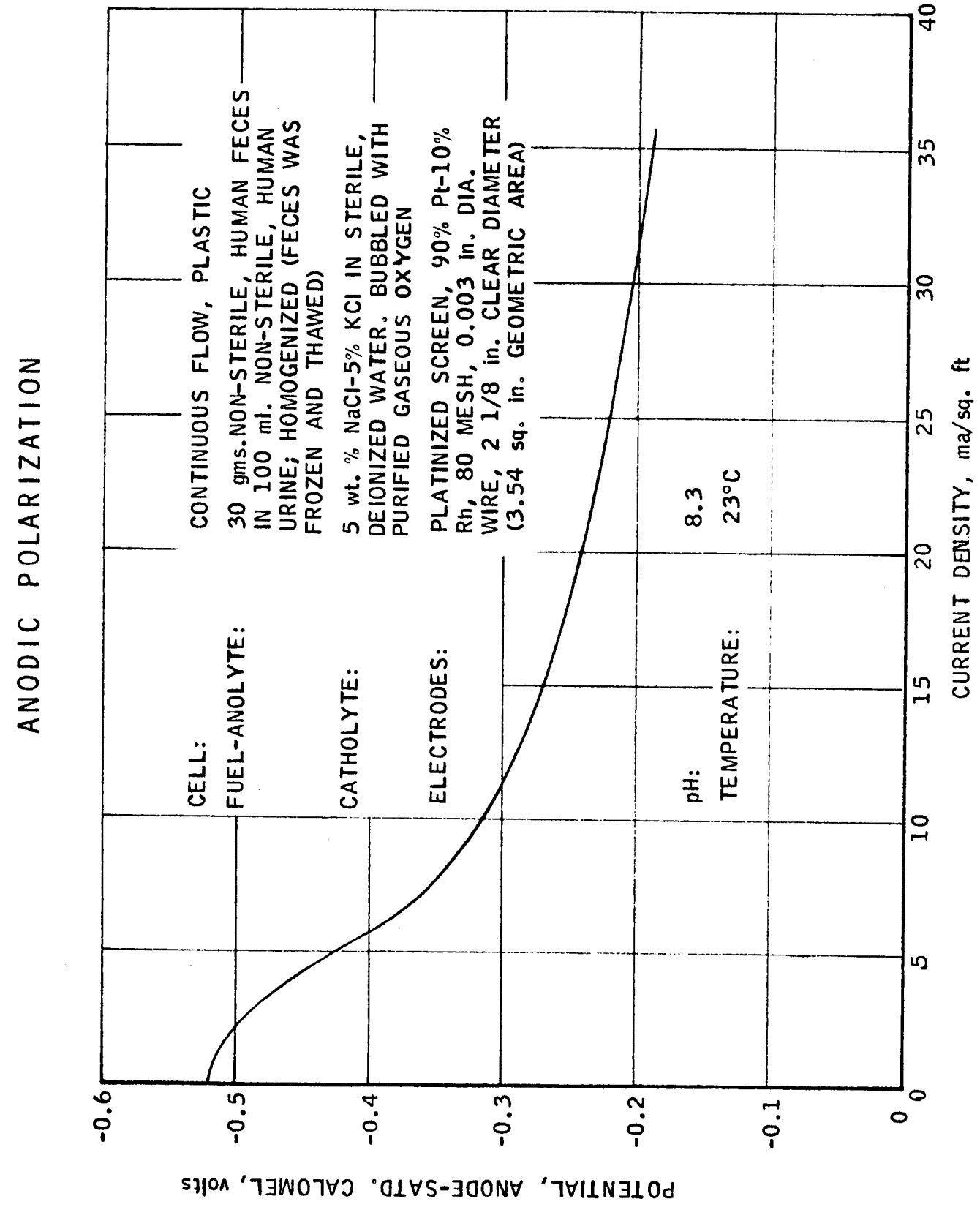
REPRODUCIBILITY STUDY USING LYOPHILIZED FECES

CELL:	H-CELL, GLASS, O-RING TYPE, NON-PUMPING
SEPARATOR:	CELLULOSE ACETATE
O-RINGS:	SILICONE
ELECTRODES:	PLATINIZED Pt FOIL, 1 sq. In. ON EACH SIDE; NON-OPPOSING FACES COATED WITH WATER RESISTANT PAINT
CATHOLYTE:	5 wt. % NaCl - 5% KCl IN STERILE, DEIONIZED WATER, WITH O_2 GAS
FUEL-ANOLYTE:	8 gms. LYOPHILIZED, HUMAN FECES, IN 100 ml. FROZEN, THAWED, AND REHEATED, NON-STERILE, HUMAN URINE; HOMOGENIZED. BUBBLED WITH PURIFIED NITROGEN



R-16,186

Figure 9



R-15,474

Figure 10

ANODIC POWER DENSITY

CELL:	CONTINUOUS FLOW, PLASTIC
ELECTRODES:	PLATINIZED SCREEN, 90% Pt - 10% Rn, 80 MESH, 0.003 in. DIA. WIRE, 2 1/8 in. CLEAR DIAMETER (3.54 sq. in. GEOMETRIC AREA)
O - RINGS:	SILICONE
SEPARATOR:	CELLULOSE ACETATE
CATHOLYTE:	5 wt. % NaCl - 5% KCl IN STERILE, DEIONIZED WATER; BUBBLED WITH PURIFIED GASEOUS OXYGEN
FUEL-ANOLYTE:	30 gms. NON-STERILE, HUMAN FECES, FROZEN AND THAWED, IN 100 ml. FRESH, NON-STERILE, HUMAN URINE. HOMOGENIZED. BUBBLED WITH GASEOUS HELIUM.
pH: 8.3	TEMPERATURE: 23°C

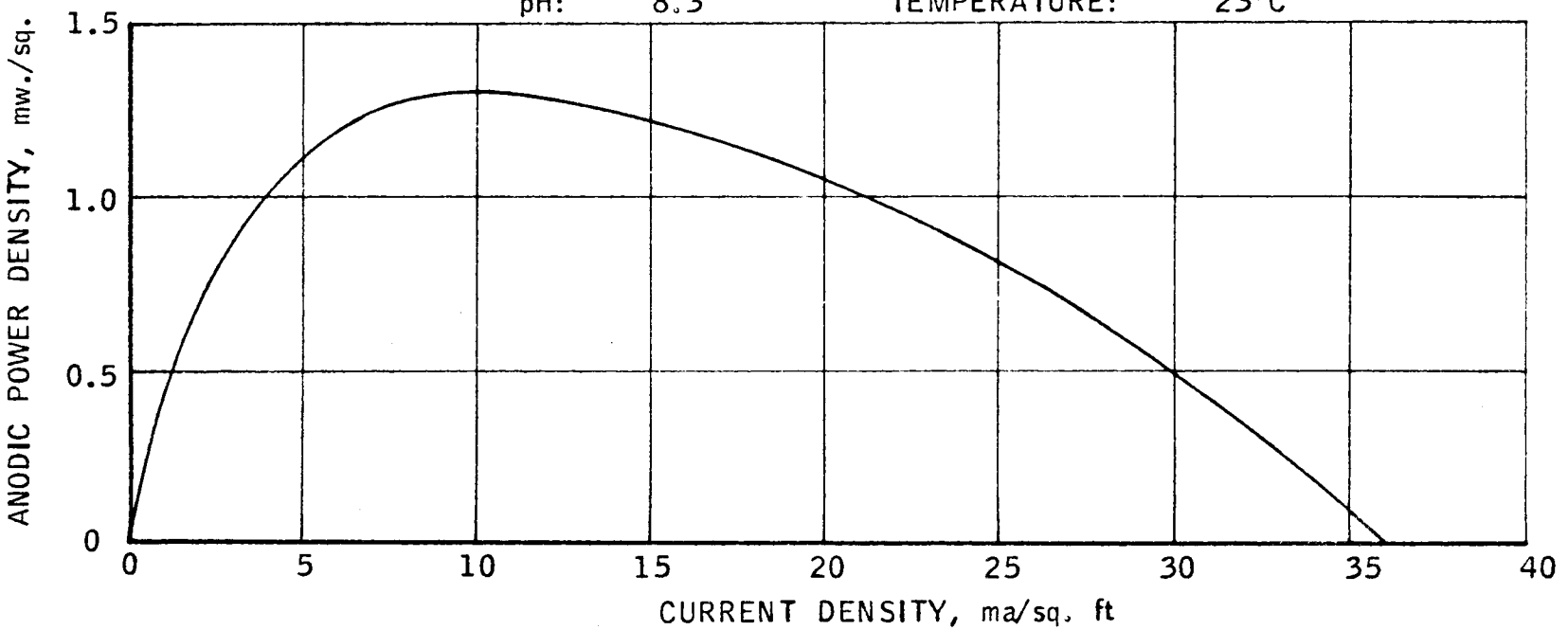


Figure 11

VARIATION OF OPEN-CIRCUIT POTENTIAL WITH TIME, CAUSED BY METABOLIC REACTIONS

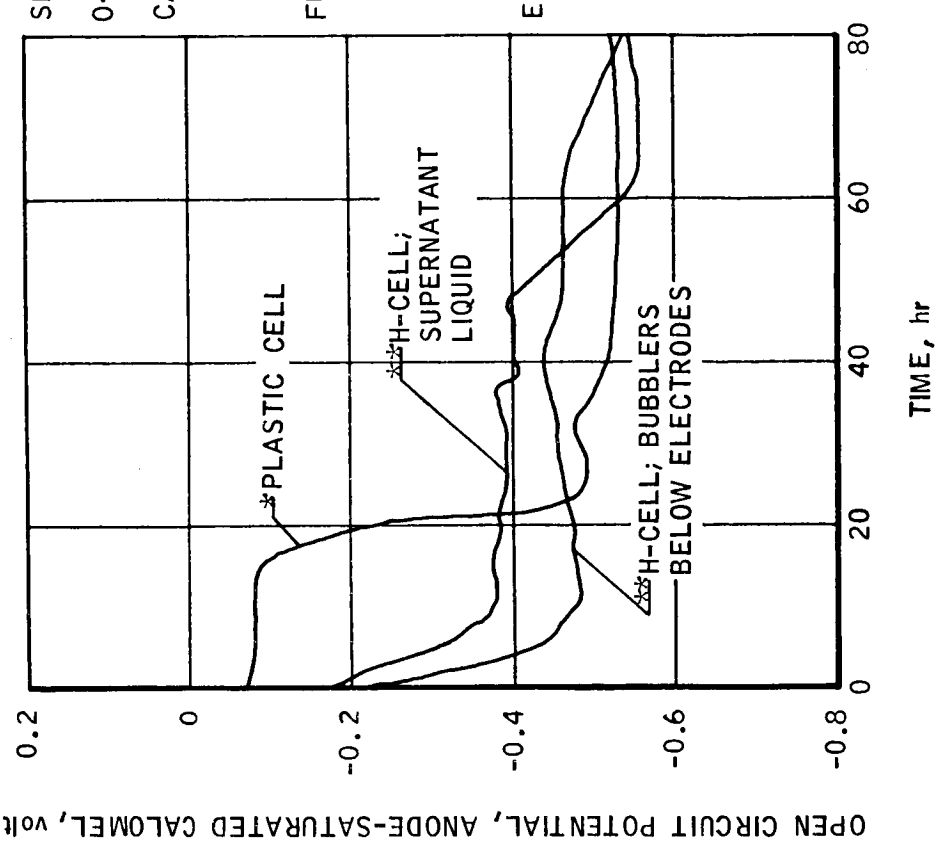
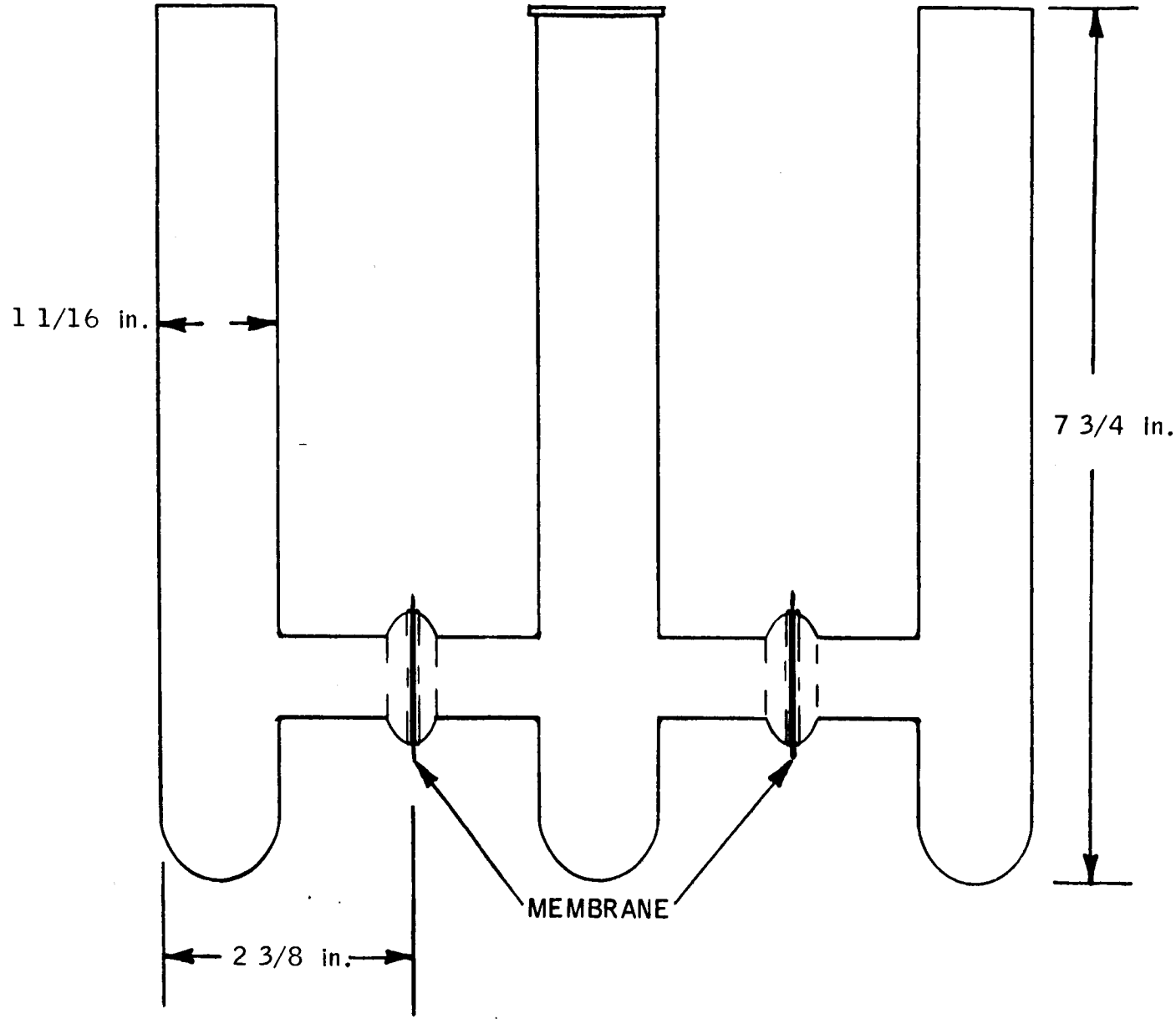


Figure 12

THREE-ARM H-CELL



R-17,033

Figure 13

REPRODUCIBILITY STUDY

URINE-FECES WITH INDIGENOUS MICRO-ORGANISMS

CELL: H-CELL, GLASS, O-RING TYPE, NON-PUMPING

SEPARATOR: CELLULOSE ACETATE

O-RINGS: SILICONE

ELECTRODES: PLATINIZED Pt FOIL, 1 sq. in. ON EACH SIDE; NON-OPPOSING FACES COATED WITH WATER RESISTANT PAINT

CATHOLYTE: 5 wt. % NaCl - 5% KCl IN STERILE, DEIONIZED WATER, WITH O_2 GAS

FUEL-ANOLYTE: 30 gms. NON-STERILE, HUMAN FECES IN 100 ml. NON-STERILE, HUMAN URINE; HOMOGENIZED. BOTH FECES AND URINE WERE PREVIOUSLY FROZEN AND THAWED

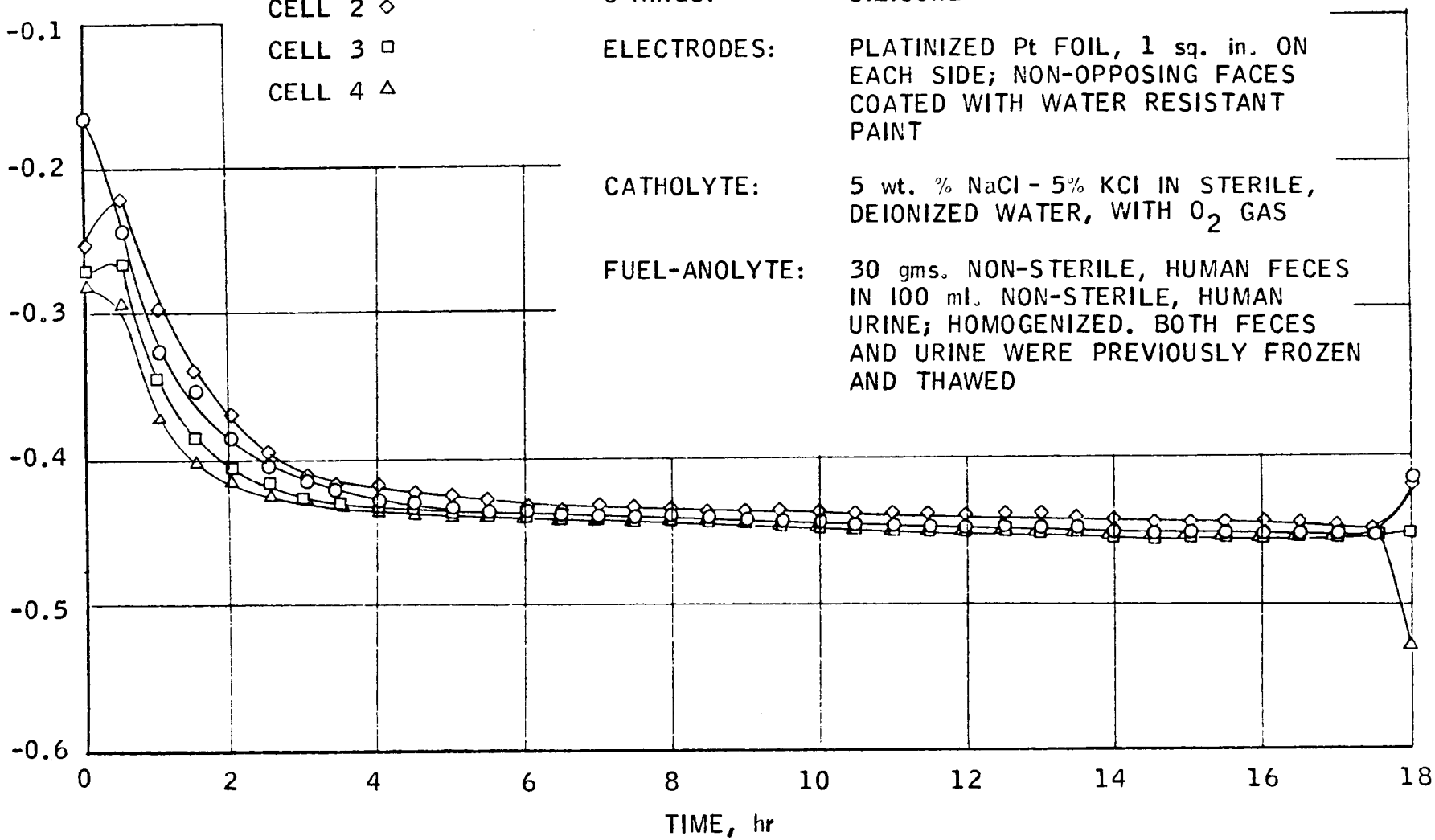


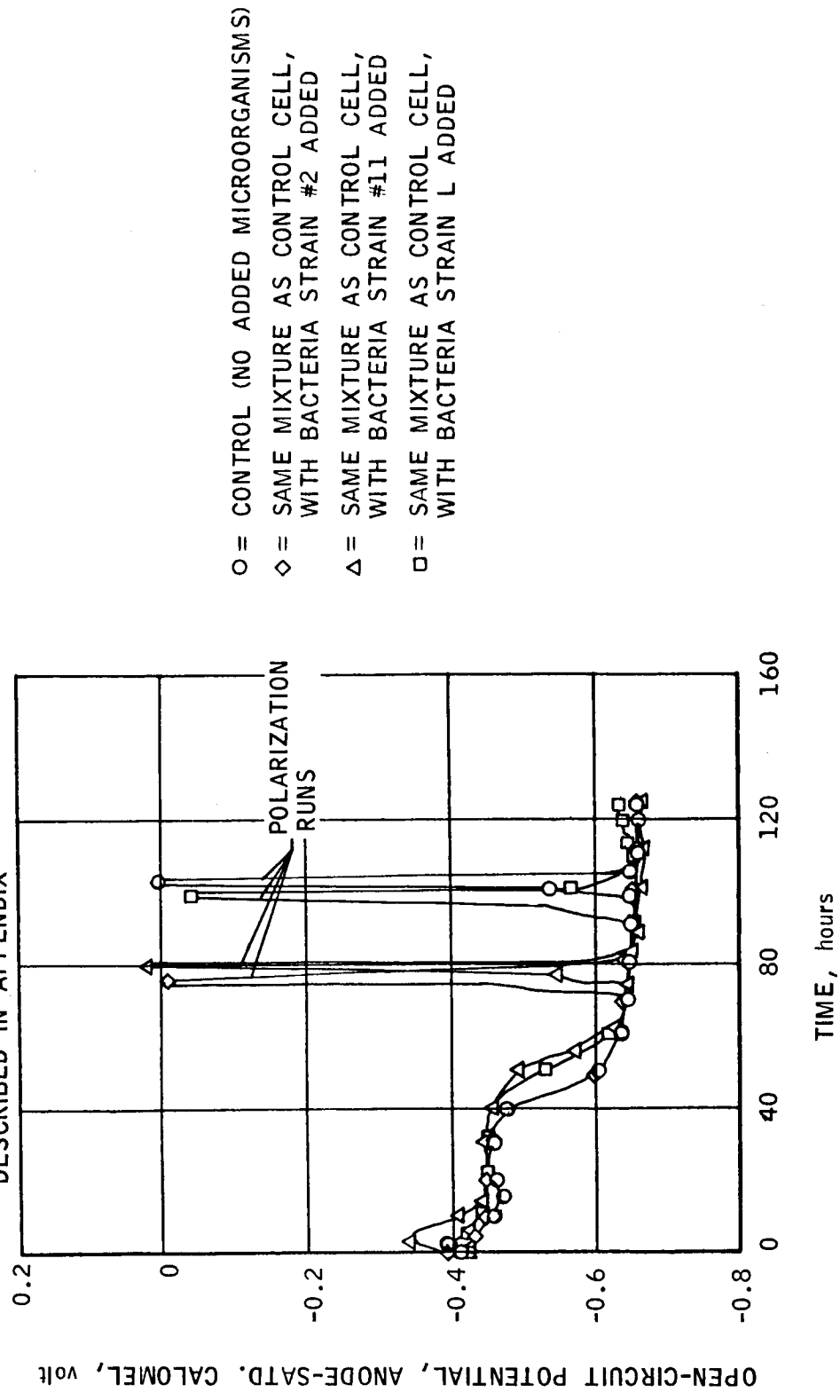
Figure 14

THE Marquardt CORPORATION VAN NUYS, CALIFORNIA

Report No. 25,144

VARIATION OF OPEN-CIRCUIT POTENTIAL WITH TIME
NONSTERILE HUMAN WASTE WITH AND WITHOUT ADDED MICROORGANISMS
ISOLATED FROM HUMAN WASTE

EXPERIMENTAL CONDITIONS ARE DESCRIBED IN TABLE I
METHODS USED IN ISOLATING THE MICROORGANISMS ARE
DESCRIBED IN APPENDIX



R-18,266

Figure 15

TABLE I

EXPERIMENTAL CONDITIONS
BIOCHEMICAL FUEL CELL

FLOW SYSTEM

Cell: Plastic (Lucite)

Electrodes: Platinized screen (90% Pt - 10% Rh), 80 mesh, 0.003 in. diameter wire, 2-1/8 in. clear diameter, 3.54 sq. in. geometric area.

Separator: Cellulose acetate (Sargent S-14825, 0.001 in. thick).

O-Rings: Silicone (Dow Corning S-7180).

Catholyte: 5 wt. % NaCl - 5% KCl in sterile, deionized water; non-biological; bubbled with purified, gaseous oxygen

Waterproof and Chemically Resistant Paint: Temprotec TP 220 Red (Ryan Herco Products Corp., Burbank, Calif.)

Fuel-Anolyte: 30 gms. non-sterile, human urine (frozen and reheated to 120°F). Feces was obtained from volunteers on a low cellulose diet, and was frozen immediately after collection. Homogenized in Osterizer Deluxe (John Oster Mfg. Co., Milwaukee). Bubbled with helium.

NON-FLOW SYSTEM

Cell: Glass, H-Shape, O-Ring type

Electrodes: Platinized platinum foil, 1 sq. in. area (non-opposing faces coated with waterproof and chemically resistant paint).

Separator, O-Rings, Catholyte, Fuel-Anolyte, and Waterproof and Chemically Resistant Paint: Same as for flow system.

TABLE II

FECES ANALYSIS AND CHARACTERISTICS*

Daily Quantity: 54 to 64 gms., meat diet; 160 to 250 gms., meat and vegetable diet; 370 gms., vegetable diet

pH: 4.6 to 8.8

Composition: 75% water; 25% solids. The primary constituents of the dry substance are bacteria (8%), salts and mucus (7%), food residues (10%), fats (6%), cellulose (2%).

*Documenta Geigy, Scientific Tables, Fifth Edition, S. Karger, New York (1959)

TABLE II (Continued)

PROXIMATE COMPOSITION OF HUMAN FECES

Component	Weight (gm.)	Per cent of Total
Bulk	150	
Water	99	66.0
Dry Matter	27	17.8
Fat	4.7	3.0
Protein	?	?
Nitrogen	1.5	1.0
Carbohydrate	?	?
Minerals	2.1	1.4
Sodium	0.12	
Potassium	0.47	
Calcium	0.64	
Magnesium	0.20	
Chloride	0.09	
Phosphorus	0.51	
Sulfur	0.13	
Trace Elements:		
Copper		
Iron		
Lead		
Manganese		
Nickel		
Zinc		
Arsenic		
Vitamins	0.015	0.01
Bile Pigments	0.15	0.1

Reference: Goldblith, S.A., and E.L.Wick, "Analysis of Human Fecal Components and Study of Methods for Their Recovery in Space Systems," Dept. of Nutrition, Food Science and Technology, Massachusetts Institute of Technology, Aeronautical Systems Division Tech. Rept. 61-419, Wright-Patterson Air Force Base, Ohio - Aug. 1961

TABLE III

URINE ANALYSIS AND CHARACTERISTICS*

Water content = 94½ - 95½%; solids = 4½ - 5½%

Volume: 1000 - 1600 ml. per 24-hour day

Specific Gravity: 1.016 - 1.022 (adult on normal diet)

pH: 4.8 to 7.4

Composition:

a) Nitrogen Content: Mostly urea, with small amounts of uric acid, creatine, creatinine, ammonia, amino-acids, hippuric acid, phenols, proteins, and others. The amino acids include arginine, aspartic acid, cystine, glutamic acid, histidine, leucine, isolencine, lysine, methionine, phenylalanine, threonine, tyrosine, valine, etc.

b) Other Components: Urine contains chlorides, sulfates, and phosphates of calcium, magnesium, sodium, potassium, and iron; vitamins.

*Documenta Geigy, Scientific Tables, Fifth Edition, S. Karger, New York (1959)

TABLE IV

EFFECT OF FECES-URINE RATIO UPON ELECTROCHEMICAL PROPERTIES OF BIOFUEL CELL

Run No.	8	8	9	8	9	8	9	9
Gms. feces/100 ml. urine	0	10	10	15	20	25	30	40
Anodic open-circuit potential at time of polarization study (volt)*	-0.220	-0.585	-0.638	-0.385	-0.564	-0.630	-0.488	-0.469
Peak anodic power density (mw./sq.ft.)	0.022	0.95	3.4	0.26	1.85	1.4	0.7	0.5
Short-circuit current density at time of polarization study (ma./sq.ft.)	2	13	30	6	26	36	24	22

*Referred to Saturated Calomel Electrode

TABLE V

POLARIZATION AND POWER DATA

Experiment No. / Described in Section	1 III.B.3.b	2 III.B.3.c	3 III.B.3.d
Peak Anodic Power Density (mw./sq.ft.)	1.3	0.33	0.15
Anodic Potential at Peak Power Density (volt)**	0.13	0.08	0.10
Current Density at Peak Power (ma./sq.ft.)	10	4.4	1.5
Peak Total Power Density (mw./sq.ft.)	-	0.59	0.22
Short Circuit Current Density (ma./sq.ft.)	36	11	4
Open Circuit Anodic Potential at time of polarization study (volt)*	-0.591	-0.435	-0.460
Best Anodic Open Circuit Potential (volt)*	-0.591	-0.475	-
pH, Initial	-	-	-
pH, Final	8.3	7.9	-
Duration of Test (hours)	68	144	
Distinguishing Variable	Standard	Urine-Feces Diluted	Supernatant Liquid Diluted

*All potentials are referred to Saturated Calomel Electrode
**Corrected so that the Potential at short-circuit is zero

TABLE V (continued)

POLARIZATION AND POWER DATA

Experiment No. Described in Section	4 III.B.2	5-Frozen III.B.4.b	5-Lyophilized III.B.4.b	6 III.B.2	7 III.B.4.c	7 III.B.4.c
Peak Anodic Power Density (mw./sq.ft.)	4.0	3.0	2.0	1.2	.21	1.5
Anodic Potential at Peak Power Density (volt)**	0.08	0.10	0.10	0.11	0.11	0.30
Current Density at Peak Power (ma./sq.ft.)	50.0	30.0	20.0	11.0	2.0	5.0
Peak Total Power Density (mw./sq.ft.)	9.2	4.8	3.6	9.0	.42	1.5
Short-Circuit Current Density (ma./sq.ft.)	105	73	42	25	5	21
Open-Circuit Anodic Potential at time of Polarization Study (volt)*	-0.660	-0.625	-0.625	(Approx.) -0.626	-0.45	-0.60
Best Anodic Open-Circuit Potential (volt)*	-	-0.65	-0.625	-0.626	-0.663	-0.691
pH, Initial	-	-	-	8.7	6.9	6.8
pH, Final	-	8.5	6.6	8.4-8.6	-	-
Duration of Test (hours)	-	-	-	50	140	140
Distinguishing Variable	E.coli added to supernatant liquid	Frozen feces	Lyophilized, frozen feces	E.coli added	Lyophilized, fresh feces	Fresh feces

*All potentials are referred to Saturated Calomel Electrode
**Corrected so that the Potential at short-circuit is zero

TABLE V (Continued)

POLARIZATION AND POWER DATA

Experiment No. Described in Section	8-I III.B.1	8-II III.B.1	8-III III.B.1	8-IV III.B.1	9-I III.B.1	9-II III.B.1	9-III III.B.1	9-IV III.B.1
Peak Anodic Power Density (mw./sq.ft.)	0.22	0.95	0.20	1.4	3.5	1.85	0.7	0.5
Anodic Potential at Peak Power Density (volt)**	0.22	0.16	0.10	0.09	0.19	0.15	0.14	0.10
Current Density at Peak Power (ma./sq.ft.)	1.0	6.0	2.0	15.0	18.0	12.0	5.0	5.0
Peak Total Power Density (mw./sq.ft.)	0.4	1.35	0.26	2.7	3.9	2.0	0.8	0.7
Short-Circuit Current Density (ma./sq.ft.)	2	13	6	36	30	26	24	22
Open-Circuit Anodic Potential at time of Polarization study (volt)*	-0.220	-0.585	-0.385	-06.30	-0.638	-0.564	-0.488	-0.469
Best Anodic Open-Circuit Potential (volt)*	-0.463	-0.635	-0.465	-0.535	-0.642	-0.697	-0.579	-0.608
pH, Initial	5.75	-	8.20	8.20	6.5	6.7	7.0	7.5
Duration of Test (hours)	208	208	208	208	184	184	184	184
Distinguishing Variable	0 gms. feces in 100 ml. urine	10 gms. feces in 100 ml. urine	15 gms. feces in 100 ml. urine	25 gms. feces in 100 ml. urine	10 gms. feces in 100 ml. urine	20 gms. feces in 100 ml. urine	30 gms. feces in 100 ml. urine	40 gms. feces in 100 ml. urine

*All potentials are referred to Saturated Calomel Electrode
**Corrected so that the Potential at short-circuit is zero

TABLE V (continued)

POLARIZATION AND POWER DATA

Experiment No. Described in Section	10 III.B.5	11-I III.L	11-II III.L
Peak Anodic Power Density (mw./sq.ft.)	1.6	-	-
Anodic Potential at Peak Power Density (volt)***	0.75	-	-
Current Density at Peak Power (ma./sq.ft.)	12.0	-	-
Peak Total Power Density (mw./sq.ft.)	2.3	-	-
Short-Circuit Current Density (ma./sq.ft.)	33	-	-
Open-Circuit Anodic Potential at time of Polarization Study (volt)**	-0.638	-	-
Best Anodic Open-Circuit Potential (volt)**	-0.719	-0.652	-0.462
pH, Initial	7.6	7.1*	6.95
Duration of Test (hours)	89	254	254
Distinguishing Variable	Vegetarian Feces	3-arm cell	Regular Cell

*pH of sterile portion; non-sterial portion was 6.95
**Referred to Saturated Calomel Electrode
***Corrected so that the Potential at short-circuit is zero

TABLE V (continued)

POLARIZATION AND POWER DATA

Experiment No. / Described in Section	42 III.N	43-I III.B.1	43-II III.B.1	43-III III.B.1	43-IV III.B.1	44-I III.L	44-II III.L	
Peak Anodic Power Density (mw./sq.ft.)	1.45	1.42	1.22	1.42	1.68	***	***	
Anodic Potential at Peak Power Density (volt)**	0.09	0.14	0.14	0.14	0.17	***	***	
Current Density at Peak Power (ma./sq.ft.)	16.5	10.5	8.5	10	10	***	***	
Peak Total Power Density (mw./sq.ft.)	2.0	1.65	1.42	1.58	1.95	***	***	
Total Potential at Peak Power Density (volt)*	0.12	0.16	0.17	0.16	0.20	***	***	
Short Circuit Current Density (ma./sq.ft.)	40	18	20	24	22	4	3	
Open-Circuit Anodic Potential at Time of Polarization Study (volt)*	-0.520	-0.533	-0.494	-0.523	-0.540	-0.265	-0.326	
Best Anodic Open-Circuit Potential (volt)*	-0.600	-0.540	-0.536	-0.685	-0.659	-0.695	-0.585	
pH, Initial	7.5	7.3	7.7	8.0	8.2	7.8	7.8(1)	8.2(2)
pH, Final	8.6	8.7	8.8	8.9	8.8	8.8	8.7(1)	8.5(2)
Duration of Test (hours)	116	138	138	138	138	169	169	
Elapsed Time of Test Prior to this Polarization Run (hours)	27	48	42	25	21	50	42	
Distinguishing Variable	-	-	-	-	-	2-arm cell	3-arm cell	

(1) Non-sterile fuel-anolyte

(2) Sterile fuel-anolyte

* Referred to Saturated Calomel Electrode

** Corrected so that the Potential at short-circuit is zero

*** Currents were so low that the power was insignificant

TABLE V (continued)

POLARIZATION AND POWER DATA

Experiment No. Described in Section	46[1] III.L	46[2] III.L	47 III.G.1
Peak Anodic Power Density (mw./sq.ft.)	0.72	2.2	0.2
Anodic Potential at Peak Power Density (volt)**	0.14	0.11	0.1
Current Density at Peak Power (ma./sq.ft.)	5.0	20.0	2.0
Peak Total Power Density (mw./sq.ft.)	0.84	3.25	0.2
Total Potential at Peak Power Density (volt)*	0.17	0.16	0.10
Short Circuit Current Density (ma./sq.ft.)	15	50	4
Open-Circuit Anodic Potential at Time of Polarization Study (volt)*	-0.570	-0.600	-0.509
Best Anodic Open-Circuit Potential (volt)*	-0.700	-0.700	-0.665
pH, Initial	7.5[3]	7.5[3]	8.0
	8.0[4]	8.0[4]	
pH, Final	-	-	
Duration of Test (hours)	213	213	374
Distinguishing Variable	3-arm H-cell	3-arm H-cell	4.29 coulombs of current withdrawn

(1) After 76 hours
(2) After 170 hours
(3) Sterile arm
(4) Non-sterile arm

* Referred to Saturated Calomel Electrode
** Corrected so that the potential at short-circuit is zero

TABLE V (continued)

POLARIZATION AND POWER DATA

Experiment No. Described in Section	51 III.C.1	51 III.C.1	51 III.C.1
Peak Anodic Power Density (mw./sq.ft.)	2.1	1.6	0.6
Anodic Potential at Peak Power Density (volt)**	0.14	0.12	0.13
Current Density at Peak Power Density (ma./sq.ft.)	14.5	13.5	4.5
Peak Total Power Density (mw./sq.ft.)	2.45	1.9	0.75
Total Potential at Peak Power Density (volt)*	0.17	0.14	0.17
Short Circuit Current Density (ma./sq.ft.)	32	33	17
Open-Circuit Anodic Potential at Time of Polarization Study (volt)*	-0.495	-0.545	-0.542
Best Anodic Open-Circuit Potential (volt)*	-0.812	-0.683	-0.683
pH, Initial	8.3	8.3	8.3
pH, Final	8.97	8.8	8.8
Duration of Test (hours)	400	400	400
(Hours Completed at Start of Test)	385	170	363
Distinguishing Variable	75°F	95-100°F	95-100°F

* Referred to Saturated Calomel Electrode
** Corrected so that the potential at short-circuit is zero

TABLE V (continued)

POLARIZATION AND POWER DATA

Experiment No. / Described in Section	55 III.M	55 III.M	55 III.M
Peak Anodic Power Density (mw./sq.ft.)	1.75	0.2	2.1
Anodic Potential at Peak Power Density (volt)**	0.14	0.08	0.14
Current Density at Peak Power (ma./sq.ft.)	12	2.5	15
Peak Total Power Density (mw./sq.ft.)	2.15	0.25	2.45
Total Potential at Peak Power Density (volt)*	0.18	0.10	0.16
Short Circuit Current Density (ma./sq.ft.)	30	6	32
Open-Circuit Anodic Potential at Time of Polarization Study (volt)*	-0.575	-0.514	-0.745
Best Anodic Open-Circuit Potential (volt)*	-0.760	-0.760	-0.810
pH, Initial	8.3	8.3	8.3
pH, Final	8.7	8.7	9.0
Duration of Test (hours)	408	408	408
Total Current withdrawn prior to this experiment (coulombs)	30	47.6	0
Elapsed Time at Start of this Test (hours)	146	339	382

* Referred to Saturated Calomel Electrode
** Corrected so that the potential at short-circuit is zero

TABLE V (continued)

POLARIZATION AND POWER DATA

Experiment No.	56				57		
Described in Section	III.N	III.N	III.N	III.N	IV.C.1	III.C.1	III.C.1
Peak Anodic Power Density (mw./sq.ft.)	1.55	3.7	2.0	0.65	0.25	**	**
Anodic Potential at Peak Power Density (volt)***	0.10	0.09	0.08	0.11	0.06	**	**
Current Density at Peak Power (ma./sq.ft.)	16	41.5	24	6.0	4.0	**	**
Peak Total Power Density (mw./sq.ft.)	2.3	4.9	2.2	0.85	0.4	**	**
Total Potential at Peak Power Density (volt)	0.14	0.12	0.09	0.14	0.1	**	**
Short Circuit Current Density (ma./sq.ft.)	38	105	52	13	10	**	**
Open-Circuit Anodic Potential at Time of Polarization Study (volt)*	-0.515	-0.523	-0.633	-0.355	-0.175	-0.375	-0.293
Best Anodic Open-Circuit Potential (volt)*	-0.585	-0.585	-0.795	-0.795	-0.856	-0.860	-0.860
pH, Initial	7.95	7.95	7.95	7.95	7.95	7.95	7.95
pH, Final	8.65	8.65	8.8	8.8	8.75	7.53	7.53
Duration of Test (hours)	328	328	328	328	352	352	352
Identifying Variable	Plastic cell; 169 hrs.	Plastic cell; 240 hrs.	H-cell; 143 hrs.	H-cell; 218 hrs.	75°F; E. coli	95-100°F; E. coli	95-100°F; Test at 75°; E. coli

* Referred to Saturated Calomel Electrode
** Currents were so low that the power was insignificant
*** Corrected so that the potential at short-circuit is zero

TABLE V (continued)

POLARIZATION AND POWER DATA

Experiment No. / Described in Section	III.C.1	III.C.1	III.C.1	III.C.1
	58			
Peak Anodic Power Density (mw./sq.ft.)	0.7	0.5	0.35	0.25
Anodic Potential at Peak Power Density (volt)***	0.14	0.12	0.10	0.07
Current Density at Peak Power (ma./sq.ft.)	5.0	4.0	3.5	3.5
Peak Total Power Density (mw./sq.ft.)	1.0	0.65	0.45	0.35
Total Potential at Peak Power Density (volt)	0.2	0.16	0.13	0.10
Short Circuit Current Density (ma./sq.ft.)	17	13	9	7
Open-Circuit Anodic Potential at Time of Polarization Study (volt)*	-0.472	-0.425	-0.475	-0.395
Best Anodic Open-Circuit Potential (volt)*	-0.603	-0.603	-0.504	-0.504
pH, Initial	7.63	7.63	7.63	7.63
pH, Final	7.2	7.2	--	--
Duration of Test (hours)	242	242	242	242
Identifying Variable	C. Spor.** 95-100°F; 57 hrs.	C. Spor.** 95-100°F; 127 hrs.	C. Spor.** Rm. Temp; 80 hrs.	C. Spor.** Rm. Temp; 131 hrs.

* Referred to Saturated Calomel Electrode
** Clostridium sporogenes
*** Corrected so that the potential at short-circuit is zero

TABLE V (continued)

POLARIZATION AND POWER DATA

Experiment No. Described in Section	59 III-Q	III-Q	III-Q	III-Q	III-Q	III-Q
Peak Anodic Power Density (mw./sq.ft.)	0.9	0.85	1.97	1.05	1.4	1.0
Anodic Potential at Peak Power Density (volt)**	0.13	0.11	0.11	0.10	0.1	0.10
Current Density at Peak Power (ma./sq.ft.)	7.0	7.5	17.5	10.0	14	10.0
Peak Total Power Density (mw./sq.ft.)	1.05	1.12	2.6	1.50	1.85	1.5
Total Potential at Peak Power Density (volt)	0.15	0.15	0.15	0.15	0.13	0.15
Short Circuit Current Density (ma./sq.ft.)	17	17	37	25	30	23
Open-Circuit Anodic Potential at Time of Polarization Study (volt)*	-0.590	-0.519	-0.650	-0.505	-0.300	-0.510
Best Anodic Open-Circuit Potential (volt)*	-0.685	-0.565	-0.685	-0.565	-0.685	-0.565
pH, Initial	8.52	8.52	8.52	8.52	8.52	8.52
pH, Final	8.9	8.4	8.9	8.4	8.9	8.4
Duration of Test (hours)	288	288	288	288	288	288
Identifying Variable	Helium 104 hrs.	Shaker 107 hrs.	Helium 200 hrs.	Shaker 203 hrs.	Helium 273 hrs.	Shaker 276 hrs.

* Referred to Saturated Calomel Electrode
** Corrected so that the potential at short-circuit is zero

TABLE V (continued)

POLARIZATION AND POWER DATA

Experiment No. / Described in Section	62-I III.N	62-II III.N	62-III III.N	64 III.K.3	64 III.K.3	64 III.K.3
Peak Anodic Power Density (mw./sq.ft.)	0.15	0.15	0.25	0.7	0.25	0.73
Anodic Potential at Peak Power Density (volt)**	0.06	0.06	0.07	0.09	0.07	0.1
Current Density at Peak Power (ma./sq.ft.)	2.5	2.5	3.5	8	3.5	7.5
Peak Total Power Density (mw./sq.ft.)	0.2	0.2	0.3	1.1	0.5	0.85
Total Potential at Peak Power Density (volt)	0.08	0.08	0.09	0.14	0.14	0.11
Short Circuit Current Density (ma./sq.ft.)	6	6	9	20	6	17
Open-Circuit Anodic Potential at Time of Polarization Study (volt)*	-0.365	-0.360	-0.370	-0.555	-0.529	-0.322
Best Anodic Open-Circuit Potential (volt)*	-0.530	-0.570	-0.545	-0.600	-0.485	-0.600
pH, Initial	8.3	8.3	8.3	8.0	8.0	8.0
pH, Final	--	--	--	--	--	--
Duration of Test (hours)	138	138	138	286	286	286
Identifying Variable	Repro-ducibility	Repro-ducibility	Repro-ducibility	Small Anode 118 hrs.	Small Cathode 261 hrs.	Small Anode 265 hrs.

* Referred to Saturated Calomel Electrode

** Corrected so that the potential at short-circuit is zero

TABLE V (continued)

POLARIZATION AND POWER DATA

Experiment No. / Described in Section	65 III.K.2	III.K.2	III.K.2	III.K.2
Peak Anodic Power Density (mw./sq.ft.)	0.4	**	0.25	0.55
Anodic Potential at Peak Power Density (volt)***	0.13	**	0.08	0.14
Current Density at Peak Power (ma./sq.ft.)	3.0	7.0	3.0	4.0
Peak Total Power Density (mw./sq.ft.)	0.65	1.3	0.5	0.65
Total Potential at Peak Power Density (volt)	0.22	0.19	0.17	0.16
Short Circuit Current Density (ma./sq.ft.)	8	15	9	10
Open-Circuit Anodic Potential at Time of Polarization Study (volt)*	-0.530	-0.515	-0.452	-0.450
Best Anodic Open-Circuit Potential (volt)*	-0.530	-0.560	-0.575	-0.630
pH, Initial	8.3	8.3	8.3	8.3
pH, Final	9.1	8.5	8.5	8.5
Duration of Test (hours)	130	130	130	130
Identifying Variable	Super-natant H-cell	Plastic Cell	Bubbler above electrode	Bubbler below electrode

* Referred to Saturated Calomel Electrode
** Calomel electrode became dry
*** Corrected so that the potential at short-circuit is zero

TABLE V (continued)

POLARIZATION AND POWER DATA

Experiment No.	66						
Described in Section	III.B.1	III.B.1	III.B.2	III.B.2	III.B.2	III.B.2	III.B.2
Peak Anodic Power Density (mw./sq.ft.)	**	**	**	**	**	**	**
Short Circuit Current Density (mw./sq.ft.)	**	**	**	**	**	**	**
Open-Circuit Anodic Potential at Time of Polarization Study (volt)*	**	**	**	-0.220	+0.130	-0.280	+0.065
Best Anodic Open-Circuit Potential (volt)*	-0.580	-0.580	-0.595	-0.220	-0.220	-0.610	-0.485
pH, Initial	7.1	7.1	7.1	6.4	6.4	7.1	7.1
pH, Final	6.1	6.1	6.1	3.6	3.6	6.1	6.1
Duration of Test (hours)	444	444	444	444	444	444	444
Identifying Variable	Feces without urine; non-sterile; 96 hrs.	Feces without urine; non-sterile; 120 hrs.	Cellulase to feces; 192 hrs.	Whatman Paper & Cellulase; 195 hrs.	Whatman Paper & Cellulase; 313 hrs.	Feces + Cellulase; 310 hrs.	Feces - Cellulase transferred; 358 hrs.

* Referred to Saturated Calomel Electrode

** Currents were so low that the power was insignificant

TABLE V (continued)

POLARIZATION AND POWER DATA

Experiment No. / Described in Section	67 III.H	67 III.H	67 III.H	67 III.H	67 III.H	67 III.H
Peak Anodic Power Density (mw./sq.ft.)	0.55	0.55	3.7	3.8	3.5	3.1
Anodic Potential at Peak Power Density (volt)**	0.14	0.07	0.15	0.15	0.15	0.17
Current Density at Peak Power (ma./sq.ft.)	4	8.0	24	25	24	18
Peak Total Power Density (mw./sq.ft.)	0.75	0.95	5.0	4.8	4.95	4.0
Total Potential at Peak Power Density (volt)	0.19	0.12	0.21	0.19	0.21	0.22
Short Circuit Current Density (ma./sq.ft.)	19	22	41	44	42	37
Open-Circuit Anodic Potential at Time of Polarization Study (volt)*	-0.200	-0.425	-0.390	-0.502	-0.390	-0.480
Best Anodic Open-Circuit Potential (volt)*	-0.460	-0.525	-0.460	-0.525	-0.460	-0.525
pH, Initial	8.5	8.5	8.5	8.5	8.5	8.5
pH, Final	8.8	8.7	8.8	8.7	8.8	8.7
Duration of Test (hours)	167	167	167	167	167	167
Elapsed Time at Polarization (hours)	29	31	101	104	149	152
Distinguishing Variable	Oxygen	Helium	Oxygen	Helium	Oxygen	Helium

* Referred to Saturated Calomel Electrode
** Corrected so that the potential at short-circuit is zero

TABLE V (continued)

POLARIZATION AND POWER DATA

Experiment No. Described in Section	68 III.K.3	III.K.3
Peak Anodic Power Density (mw./sq.ft.)	0.5	0.9
Anodic Potential at Peak Power Density (volt)**	0.05	0.1
Current Density at Peak Power (ma./sq.ft.)	9	9
Peak Total Power Density (mw./sq.ft.)	0.9	1.3
Total Potential at Peak Power Density (volt)	0.1	0.14
Short Circuit Current Density (ma./sq.ft.)	23	25
Open-Circuit Anodic Potential at Time of Polarization Study (volt)*	-0.425	-0.372
Best Anodic Open-Circuit Potential (volt)*	-0.675	-0.615
pH, Initial	8.5	8.5
pH, Final	8.8	8.8
Duration of Test (hours)	86	86
Distinguishing Variable	1/2 anode	1/4 anode

* Referred to Saturated Calomel Electrode
** Corrected so that the potential at short-circuit is zero

TABLE V (Continued)

POLARIZATION AND POWER DATA

Experiment No. Described in Section	69 III.R.2	69 III.R.2	69 III.R.2	70 III.R.2	70 III.B.4.a	70 III.B.4.a
Peak Anodic Power Density (mw./sq.ft.)	1.55	7.6	*	*	0.6	**
Anodic Potential at Peak Power Density (volt)****	0.11	0.17	*	*	0.15	**
Current Density at Peak Power (ma/sq.ft.)	14	45	*	*	4	**
Peak Total Power Density (mw./sq.ft.)	2.32	10.8	*	*	0.8	**
Total Potential at Peak Power Density (volt)	0.17	0.24	*	*	0.2	**
Short Circuit Current Density (ma./sq.ft.)	29	90	*	*	14	**
Open-Circuit Anodic Potential at Time of Polarization Study (volt)***	-0.638	-0.670	-0.652	-0.640	-0.450	-0.490
Best Anodic Open-Circuit Potential (volt)***	-0.665	-0.670	-0.662	-0.665	-0.643	-0.613
pH, Initial	7.8	7.8	7.8	7.8	8.65	8.3
pH, Final	-	-	-	8.5	8.85	8.65
Duration of Test (hours)	122	122	122	122	150	150
Distinguishing Variable	Added Strain #2	Added Strain L	Control	Added Strain #11	Fresh Urine	Frozen-Thawed Urine

* Erroneously high values, reason unknown
** Erroneously low values, reason unknown
*** Referred to Saturated Calomel Electrode
**** Corrected so that the potential at short-circuit is zero.

TABLE V (Continued)

POLARIZATION AND POWER DATA

Experiment No. / Described in Section	73 III.R.2	73 III.R.2	73 III.R.2	75 III.K.3
Peak Anodic Power Density (mw/sq.ft.)	0.9	0.69	0.98	0.22
Anodic Potential at Peak Power Density (volt)**	0.2	0.17	0.12	0.22
Current Density at Peak Power (ma/sq.ft.)	4.0	4.0	8.0	1.0
Peak Total Power Density (mw/sq.ft.)	1.35	0.84	1.24	0.22
Total Potential at Peak Power Density (volt)	0.33	0.21	0.16	0.22
Short Circuit Current Density (ma/sq.ft.)	15	12	14	5
Open-Circuit Anodic Potential at Time of Polarization Study (volt)*	-0.650	-0.472	-0.527	-0.501
Best Anodic Open Circuit Potential (volt)*	-0.650	-0.615	-0.540	-0.580
pH, Initial	8.3	8.3	8.3	7.3
pH, Final	8.9	8.9	8.6	8.3
Duration of Test (hours)	186	186	186	170
Distinguishing Variable	Bacteria 2	Bacteria 11 from Expt. 69	New Bacteria 11	1/8 anode

* Referred to Saturated Calomel Electrode
** Corrected so that the potential at short-circuit is zero.

TABLE VI

Pretreatment of Human Waste
by Addition of Nutrients

Open-circuit potentials measured were anode versus cathode.

P-1

Time (hours)	0	24	48	120
Open-circuit potential (v.)	0.145	0.638	0.475	0.425

P-2

Time (hours)	0	24	48	120	144	168
Open-circuit potential (v.)	0.148	0.660	0.480	0.580	0.475	0.600

P-3

Time (hours)	0	72	96	120
Open-circuit potential (v.)	0.175	0.470	0.140	0.120

P-4

Time (hours)	0	24	68	96	120
Open-circuit potential (v.)	0.290	0.240	0.260	0.290	0.270

P-5a

Time (hours)	0	24	48	144	168	192	216
Open circuit potential (v.)	0.110	0.210	0.730	0.590	0.525	0.350	0.290

TABLE VI (continued)

P-5b

Time (hours)	0	24	48	144	168	192	216
Open-circuit potential (v.)	0.110	0.360	0.475	0.210	0.110	0.130	0.085

P-5c

Time (hours)	0	24	48	144	168	192	216
Open-circuit potential (v.)	0.110	0.550	0.325	0.300	0.450	0.445	0.440

P-6a

Time (hours)	0	24	48	72	96	168	192	216
Open-circuit potential (v.)	0.140	0.760	0.835	0.680	0.680	0.525	0.250	0.125

P-6b

Time (hours)	0	24	48	72	96	168	192	216
Open-circuit potential (v.)	0.160	0.285	0.450	0.540	0.420	0.580	0.440	0.575

P-7

Time (hours)	0	27	51	148	168
Open-circuit potential (v.)	0.520	0.550	0.425	0.450	0.400

TABLE VII

ELECTROLYTIC RESISTIVITIES OF SELECTED ELECTROLYTES (ohms)
(Each value is an average of five readings)

	First Test Frequency (cps)		Second Test Frequency (cps)		
	400	1000	400	1000	10,000
Saturated KCl	--	--	3.1	3.4	3.4
0.1 N KCl	143.7	145.2	148.8	148.4	145.3
2.5% NaCl by weight + 2.5% KCl	23.59	23.56	23.34	23.06	--
5% NaCl by weight + 5% KCl	13.21	13.21	13.24	13.56	13.48
Frozen urine, filtered	94.05	93.53	--	--	--
Fresh urine	59.81	59.10	--	--	--
Urine, frozen then thawed at room temperature	92.2	95.3	92.2	95.3	96.0
Deionized water			Exceeds 2×10^6		

TABLE VIII

ELECTROLYTIC RESISTIVITY OF SEPARATORS (ohms)
(Each value is an average of five readings)

	First Test Frequency (cps)			Second Test Frequency (cps)		Avg. Resistance of Separator at 400 cps (ohms)	Specific Resistivity (ohm-cm.)
	400	1000	10,000	400	1000		
Satd. KCl, after soaking separators	3.102	3.001	3.316	5.409	5.199	--	--
Cellulose acetate in the above satd. KCl	2976*	2435*	497*	1913*	1730*	--	--
Anion exchange membrane in the above satd. KCl	3.360	3.575	3.814	5.478	5.363	0.163	33.6
Cation exchange membrane in the above satd. KCl	3.56	3.66	3.92	5.408	5.296	0.229	47.2
Same satd. KCl, after tests	3.31	3.45	3.53	5.416	5.274	--	--
Satd. KCl, after soaking dialysis membrane	5.240	5.178	--	--	--	--	--
Dialysis membrane in the above satd. KCl	5.285	5.202	--	--	--	.045	79.6
Same satd. KCl, after tests	5.251	5.179	--	--	--	--	--

Cellulose acetate was E.H. Sargent Co. S-14825, 0.001 in. thick (.0025 cm.)
Anion exchange membrane was Ionics, Inc., Nepton AR-111A, 0.024 in. thick (.061 cm.)
Cation exchange membrane was Ionics, Inc., Nepton CR-61, 0.024 in. thick (.061 cm.)
Dialysis membrane was VanWaters and Rogers, Inc., No. 25225, 0.0028 in. thick (.0071 cm.)

*These values are believed to be excessively high, but the reason for them has not been determined.

Cross-sectional area of all membranes = 12.6 sq.cm.(40 mm. I.D.)

Deionized water was used to make the electrolytes.

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TABLE IX

CELL CONSTANTS

Electrolyte: Saturated KCl solution

Temperature: 25°C

	Frequency (cycles/sec)	Resistance (ohms)	Cell Constant ($cm.^{-1}$)
Conductivity	400	5.223	2.13
	1000	5.047	2.06
H-Cell, O-Ring Type	400	4.931	2.01
	1000	4.895	1.99
H-Cell, Sintered	400	4.847	1.98
Plug Type	1000	4.734	1.93
H-Cell, Sintered	400	4.912	2.00
Plug with Agar	1000	4.804	1.96

TABLE X

STATISTICAL DATA OBTAINED FROM REPRODUCIBILITY TESTS WITH VARIOUS FUELS
Based on Anodic Open-Circuit Potentials (millivolts)

Phase	I	II	III	IV	V
Mean maximum difference (millivolts)	16.49	20.36	40.50	9.04	37
Mean deviation of maximum differences	9.58	10.213	31.84	.96	3.0
Standard deviation of maximum differences	11.63	18.09	37.44	1.19	3.55

VARIATION OF STATISTICAL VALUES WITH TIME, PHASE V

	Four cells, 50 hours	Four cells, 24 hours	Three cells, 24 hours
Variance	423	89	12.6
Standard deviation	11.1	9.4	3.55
Mean deviation	±16	± 8	± 3
% Error	43.2	30.7	25.0
Mean	37	26	12

TABLE XI

STATISTICAL DATA FROM REPRODUCIBILITY TESTS

Experiment No.	39	40	41	42
Type of System	Flow	Non-flow	Flow	Flow
No. of Cells	2	3	2	2
Mean of Maximum Differences of Potentials (millivolts)	113.6	114.0	19.5	27.5
Mean Deviation	51.1	21.4	10.6	35.2
Variance	3706	1024	155	3391.6
Standard Deviation	60.9	32.0	12.4	58.2

TABLE XII

CALCULATION OF HEATING VALUE OF LYOPHILIZED, HUMAN FECES

m = weight of sample (1.0200 gms.; Residue = 0.0803; Consumed = 0.9397 gms.)

t_a = temperature at time of firing: 28.40°C (83.12°F)

t_f = final maximum temperature: 30.20°C (86.36°F)

c_1 = ml of base required for acid titration: 17.00 ml; normality of base used: 0.0725

c_2 = percentage of sulfur in sample: 0.5905%

c_3 = centimeters of fuse wire used: 3.4 cm

w = energy equivalents of calorimeter in calories per degree centigrade: 2415 cal/°C

E_1 = correction in calories for heat formation of nitric acid (HNO_3) (1 cal/ml) = c_1 if .0725 N alkali was used for acid titration: 17.00

E_2 = correction in calories for heat of formation of sulfuric acid (H_2SO_4): (14) (c_2) (m): 7.77 cal

E_3 = correction in calories for heat of combustion of fuse wire 2.3 cal/cm: 8.0 cal

$t = t_f - t_a$ = net temperature rise: 1.80°C

$$H = \frac{t\,w - E_1 - E_2 - E_3}{m} = 4591 \text{ cals./gm.}$$

	Bomb Temp.	Jacket Temp.
Initial Temperature	82.25°F	82.25°F
Final Temperature	86.36°F	86.36°F

TABLE XII (Continued)

m = weight of sample (0.9224 gms.; Residue = 0.0758 gms.; Consumed = 0.8466 gms.)

t_a = temperature at time of firing: 26.90°C (80.42°F)

t_f = final maximum temperature: 28.58°C (83.44°F)

c_1 = ml of base required for acid titration: 15.75 ml; normality of base used: 0.0725

c_2 = percentage of sulfur in sample: 0.5808%

c_3 = centimeters of fuse wire used: 4.35 cm

w = energy equivalents of calorimeter in calories per degree centigrade: 2415 cal/°C

E_1 = correction in calories for heat formation of nitric acid (HNO_3) (1 cal/ml) = c_1 if .0725 N alkali was used for acid titration: 15.75

E_2 = correction in calories for heat of formation of sulfuric acid (H_2SO_4): (14) (c_2) (m): 6.88 cal

E_3 = correction in calories for heat of combustion of fuse wire 2.3 cal/cm: 10.0 cal.

$t = t_f - t_a$ = net temperature rise: 1.66°C

$$Hg = \frac{t\,w - E_1 - E_2 - E_3}{m} = 4697 \text{ cals./gm.}$$

	Bomb Temp.	Jacket Temp.
Initial Temperature	80.42°F	80.42°F
Final Temperature	83.44°F	83.44°F

TABLE XIII
COMPOSITIONS OF MEDIA

Lactate Medium
(Basis: 1 liter of medium)

$MgSO_4 \cdot 7H_2O$	0.2 gm
K_2HPO_4	1.0
$FeSO_4 \cdot 7H_2O$	50.0
$CaCl_2$	20.0
$MnCl_2 \cdot 2H_2O$	2.0
$NaMoO_4 \cdot 2H_2O$	1.0
NH_4Cl	1.0
KNO_3	1.0
$CaCO_3$	2.0
Na lactate	2.0

TABLE XIII (Continued)

Liquid Starkey Medium

(The following ingredients are added to 500 milliliter tap water)

Peptone	2.5 gm
Beef extract	1.5
Yeast extract	0.1
$MgSO_4 \cdot 7H_2O$	1.5
Na_2SO_4	0.75
$Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$	0.1
Glucose (dextrose)	2.5

TABLE XIII (Continued)

Sulfate Medium
(Basis: 1 liter of medium)

$MgSO_4 \cdot 7H_2O$	0.2	gm
K_2HPO_4	1.66	
KH_2PO_4	1.0	
$FeSO_4 \cdot 7H_2O$	0.005	
$MnCl_2 \cdot 2H_2O$	0.002	
$HmoO_4$	0.001	
KNO_3	1.0	
Na lactate	5.0	
$CaCl_2$	0.002	
Na_2SO_4	1.0	
NH_4Cl	1.0	

TABLE XIII (Continued)

Nitrate Medium
(Basis: 1 liter of medium)

$MgSO_4 \cdot 7H_2O$	0.2 gm
K_2HPO_4	1.66
KH_2PO_4	1.0
$FeSO_4 \cdot 7H_2O$	0.005
$MnCl_2 \cdot 2H_2O$	0.002
$HMoO_4$	0.001
KNO_3	1.0
Na lactate	5.0
$CaCl_2$	0.002

TABLE XIII (Continued)

D-17 Medium
(Basis: 3 liters of medium)

The medium consists of the following eight stock solutions:

1) Sea Water - 2.25 liters, Distilled Water - 511 ml, EDTA - 12 ml of a stock solution which is 50 gm/l in the di-sodium salt. Prepared in a one gallon autoclave bottle.

2) $MgSO_4 \cdot 7H_2O$--7.5 gm in 25 ml Dist. H_2O.

3) KNO_3--6.0 gm in 50 ml Dist. H_2O.

4) KH_2PO_4--3.0 gm in 25 ml Dist. H_2O.

5) K_2HPO_4--3.0 gm in 25 ml Dist. H_2O.

6) $CaCl_2$--0.325 gm in 25 ml Dist. H_2O.

7) H_3BO_3--0.342 gm; $Fe_2(SO_4)_3$ 0.015 gm in 25 ml H_2O.

8) 60 ml of a solution containing:

$ZnSO_4 \cdot 7H_2O$--4.410 gm/l

$MnCl_2 \cdot 4H_2O$--0.700 gm/l

H_2MoO_4 (85%)--0.462 gm/l

$CuSO_4 \cdot 5H_2O$--0.800 gm/l

$Co(NO_3)_2$--0.250 gm/l

The stock solutions are autoclaved separately and allowed to cool to room temperature. Then each solution is added aseptically to solution No. 1, slowly and with vigorous agitation.

APPENDIX B

CALCULATIONS OF CELL CONSTANTS

The cell constants were calculated from the following equations:

$$L = \frac{Kd}{A}$$

$$L = \frac{\Lambda c}{1000}$$

$$L = JK = J/R = \frac{d}{AR}$$

$$L = 1/k$$

L = specific conductance of the solution (mho/cm.)

k = specific resistance of the solution (ohm-cm.)

K = conductivity of the solution (mho)

d = distance between electrodes (cm.)

A = cross section of electrolytic path (sq.cm.)

Λ = equivalent conductance of solution (mho/equivalent/sq.cm.)

c = concentration of solution (equivalents/liter)

J = cell constant ($cm.^{-1}$)

R = measured resistance (ohms)

Equivalent conductivities (Λ) and specific conductivities (L) of various electrolytes may be obtained from several sources (2-5), and the specific resistance (k) can be calculated from them. For example, the specific resistance of saturated potassium chloride, used in obtaining the cell constants tabulated in Table IX , is 2.45 ohm-cm. Using this value and measuring the resistances of the various cells, the data of Table IX were obtained.

The technique used in making these determinations was that of four-electrode conductometry.(1) Briefly, in this procedure four electrodes were used; two platinized platinum foil electrodes were inserted in the conductivity cell shown in Figure 6 to carry the current, and two saturated calomel reference electrodes were placed between the two platinum electrodes. The conductivity cell is relatively long and narrow to provide essentially parallel electrolytic paths. The reference electrodes were placed at the upper edge of the platinum electrodes to avoid shielding.

APPENDIX C

DESCRIPTION OF METHODS USED IN ISOLATING MICROORGANISMS FROM HUMAN WASTE

Strain L:

A mixture of non-sterile, human urine and feces was prepared, containing 30 grams feces in 100 milliliters of urine. The mixture was centrifuged to remove solids.

Agar was prepared in 0.9 weight percent sodium chloride solution, and then sterilized. The agar mixture was added to the supernatant liquid obtained by centrifuging the human waste and then poured on plates. The agar plates were then incubated at 37°C for 24 hours.

The colonies which grew on agar were streaked on trypticase soy agar incubated at 37°C for 24 hours, then refrigerated.

Strain No. 2:

Plates were prepared by mixing equal volumes of non-sterile urine or urine-feces with 3 weight percent agar containing 1 weight percent sodium chloride. Half of the urine plates were streaked with a non-sterile emulsion of feces. Therefore, there were three kinds of agar plates; one contained urine-feces mixture, another contained feces streaked on urine, and the third contained urine only.

These plates were allowed to stand at room temperature for four days, half of the plates under aerobic conditions and half under anaerobic conditions. Then colonies were taken from the plates, added to trypticase soy broth, and stained.

The strain designated as No. 2 was obtained from a urine-agar plate streaked with feces and maintained aerobically. A large colony grew, which resembled Bacillus and had large, Gram-positive rods in chains.

Slants were prepared of this organism, and when streaked on sterile urine and urine-feces plates, growth was visible after 24 hours. Growth was better on the urine-feces plates.

Strain No. 11:

A sample of contaminant was removed from glass, H-cells which had supposedly been sterilized. The contaminant was transferred to trypticase soy agar, and incubated at 37°C. The "contaminant" grew and was pink in color, and was composed of Gram-positive large rods and ovals.

APPENDIX D

SUMMARY OF PRELIMINARY EXPERIMENTS

The results of some of the early screening experiments are presented below. In each case, the type of separator and cell used, the media employed as fuel-anolyte mixture and catholyte, and the microorganisms added (if any) are described. Formulae for the appropriate media are listed in Tables II through VI; all media were sterile. Feces and urine were of human origin and non-sterile unless otherwise indicated.

All readings of current were of short duration (less than two seconds) and were intermittent; they were not steady-state values under sustained loads. Therefore, instead of recording actual current densities, the current density data have been categorized arbitrarily, with values ranging from I to III (highest current densities are in Category I). The best open-circuit potential obtained from each system is also presented.

Glass H-Cells with Sintered Glass-Agar Plugs; Platinized Platinum Foil Electrodes

Experiment 1: Fuel-anolyte contained Desulfovibrio desulfuricans, 3 percent feces, 28 percent triple filtered sea water (final filtration was with 0.45μ Millipore filter paper), and 69 percent lactate medium. Anode mixture was pre-incubated at 37°C for 3 days. (Catholyte was lactate medium. (Category II; 0.640 v.)

Experiment 2: Essentially the same as Experiment 1, except that the catholyte was liquid Starkey medium. Both anode and cathode mixtures were pre-incubated at 37°C for 3 days. (Category III; 0.510 v.)

Experiment 3: Essentially the same as Experiment 1, except that the added electrolyte was sulfate medium. The catholyte was nitrate medium; a microorganism was used (Escherichia coli). Both anode and cathode mixtures were pre-incubated at 37°C for 3 days. (Category III; 0.430 v.)

Experiment 4: Essentially the same as Experiment 3, except that the cathode microorganism was Bacillus subtilis. (Category III, 0.580 v.)

Experiment 5: Fuel-anolyte contained 10 percent (by weight) feces, 10 percent urine, and 80 percent triple filtered sea water (final filtration was with 0.45μ Millipore filter paper). (Category III; 0.680 v.)

Experiment 6: Fuel-anolyte contained 5 percent feces, 5 percent urine, and 90 percent sulfate medium. The pH was maintained at 6.0 to 7.0. Catholyte was sulfate medium. (Category II; 0.840 v.)

Experiment 7: Essentially the same as Experiment 6, except that air was bubbled continuously through the mixture. The mixture was maintained at 42.5°C, except that electrical properties were obtained at room temperature. (Category III; 0.650 v.)

Experiment 8: Fuel-anolyte contained 5 percent (by weight) feces, 5 percent urine, and 90 percent sterile lactate medium, and was incubated at 37°C for 24 hours. Catholyte was lactate medium.(Category III; 0.720 v.)

Experiment 9: Same as Experiment 8, except that sterile liquid Starkey medium was used instead of lactate medium. (Category III; 0.530 v.)

Experiment 10: Same as Experiment 8, except that sterile distilled water was used instead of lactate medium. (Category III; 0.610 v.)

Experiment 11: Same as Experiment 8, except that triple filtered sea water (final filtration with 0.45μ Millipore filtered paper) was used instead of lactate medium. (Category III; 0.650 v.)

Experiment 12: Fuel-anolyte mixture contained feces and urine in the weight ratio 3:4, to provide a 1 percent (by weight) fuel mixture, and Escherichia coli was added. The medium was D-17. (Category III; 0.575 v.)

Experiment 13: Same as Experiment 12, except that a cathode micro-organism was used (Escherichia coli). (Category III; 0.176 v.)

Experiment 14: Same as Experiment 12, except that no Escherichia coli was added to the fuel-anolyte. (Category III; 0.075 v.)

Experiment 15: Same as Experiment 14, except that a cathode micro-organism was used (Escherichia coli). (Category III; 0.180 v.)

Experiment 16: Fuel-anolyte mixture contained Desulfovibrio desulfuricans (DSV), 1 percent feces, 1 percent urine, and 98 percent sulfate media. A cathode micro-organism (Escherichia coli) was used in sterile nitrate medium. (Category I; 0.740 v.)

Experiment 17: Same as Experiment 16, except without feces in the fuel-anolyte mixture. (Category I; 0.700 v.)

Experiment 18: Same as Experiment 16, except without urine in the fuel-anolyte mixture. (Category II; 0.650 v.)

Experiment 19: Same as Experiment 16, except without cathode micro-organism. (Category II; 0.650 v.)

Experiment 20: Fuel-anolyte mixture contained Escherichia coli, 1 percent feces, and 99 percent sterile sulfate medium. The cathode mixture was sterile nitrate medium without micro-organisms. (Category II; 0.865 v.)

Experiment 21: Fuel-anolyte mixture contained 1 percent feces, 1 percent urine, and the fungus Linderina in sterile sulfate medium. The

cathode was nitrate medium. (Category III; 0.700 v.)

Experiment 22: Same as Experiment 21, except without urine. (Category III; 0.430 v.)

Experiment 23: Same as Experiment 21, except without feces. (Category III; 0.258 v.)

Experiment 24: Fuel-anolyte mixture contained 1 percent feces (by weight) and Escherichia coli in sulfate medium; catholyte was nitrate medium. (Category III; 0.430 v.)

Experiment 25: Same as Experiment 24, except that the fuel-anolyte mixture was pre-incubated at 37°C for 24 hours.(Type III; 0.385 v.)

Experiment 26: Same as Experiment 24, except that the feces was sterilized before being added to the sulfate medium. (Category I; 0.675 v.)

Experiment 27: Same as Experiment 24, except that the feces-sulfate mixture was sterilized after homogenizing. (Category II; 0.780 v.)

Experiments 28 and 29: Same as Experiment 26, except that greater care was used in maintaining sterility. (Category II; 0.720 and 0.675 v., respectively.)

Experiment 30: Fuel-anolyte contained 10 percent (by weight) feces in urine; catholyte was nitrate medium. (Category I; 0.730 v.)

Experiment 31: Same as Experiment 30, except 0.1 percent (by weight) each of Na_2SO_4 and NH_4Cl were added to the fuel-anolyte. (Category II; 0.720 v.)

Experiment 32: Same as Experiment 30, except that sulfate medium was used instead of urine. (Category III; 0.630 v.)

Experiment 33: Same as Experiment 30, except that the fuel-anolyte contained 65 percent (by weight) sulfate medium and 25 percent triple filtered sea water (final filtration with 0.45μ Millipore filter paper) instead of urine. (Category III; 0.600 v.)

Experiment 34: Same as Experiment 30, except that the fuel-anolyte contained 90 percent (by weight) sulfate medium and Escherichia coli instead of urine. (Category III; 0.650 v.)

Experiment 35: The fuel-anolyte was urine, the catholyte nitrate medium. (Category III; 0.260 v.)

Experiment 36: Fuel-anolyte was urine, to which 0.1 percent (by weight) each of Na_2SO_4 and NH_4Cl were added. The catholyte was nitrate medium. (Category III; 0.680 v.)

Experiment 37: Fuel-anolyte was 10 percent (by volume) urine in 90 percent sulfate medium. The catholyte was nitrate medium. (Category I; 0.825 v.)

Experiment 38: Fuel anolyte contained 10 percent (by weight) urine, 25 percent triple filtered sea water (final filtration through 0.45μ Millipore paper), and 65 percent sulfate medium. The catholyte was nitrate medium. (Category II; 0.735 v.)

Experiment 39: Fuel-anolyte contained 10 percent (by weight) urine, 90 percent sulfate medium, and Escherichia coli; catholyte was nitrate medium. (Category III; 0.635 v.)

Experiment 40: Same as Experiment 36, except that 1 milliliter of Pseudomonas denitrificans was added to 100 milliliters of urine. (Category III; 0.630 v.)

Experiment 41: Fuel-anolyte contained 10 percent (by weight) feces in urine; the agar plug was made in saturated KCl solution; and the catholyte was 5 percent KCl and 5 percent NaCl in deionized water. (Category II; 0.745 v.)

Experiment 42: Same as Experiment 41, except that the agar plug was made in the catholyte solution. (Category II; 0.730 v.)

Experiment 43: Same as Experiment 41, except that the fuel-anolyte contained 20 percent feces. (Category II; 0.700 v.)

Experiment 44: Same as Experiment 43, except that the agar plug was made in the catholyte solution. (Category II; 0.700 v.)

Experiment 45: Same as Experiment 41, except that the fuel-anolyte contained 30 percent feces. (Category II; 0.630 v.)

Experiment 46: Same as Experiment 45, except that the agar plug was made in the catholyte solution. (Category I; 0.755 v.)

Plastic, Continuous Flow System; Platinized Platinum Screen Electrodes

Experiment 47: Fuel-anolyte contained 25 percent (by weight) feces, 25 percent urine, and 50 percent sulfate medium. Catholyte was non-biological (air) in sulfate medium. Separator was cellulose acetate.(1) (Category III; 0.370 v.)

Experiment 48: Same as Experiment 47, except for the separator.

a) Separator was cellulose acetate.(1) (Category III; 0.425 v.)
b) Separator was anion exchange membrane.(2) (Category II; 0.750 v.)

Experiment 49: Same as Experiment 48, to check reproducibility. (Category III; 0.450 v., and Category III; 0.380 v., respectively.)

Experiment 50: Same as Experiment 47, except for the separator.

a) Separator was cellulose acetate.(1) (Category III; 0.500 v.)
b) Separator was cation exchange membrane.(3) (Category II; 0.600 v.)

(1) Cellulose acetate was E. H. Sargent Co. S-14825, 0.001 inch thick.

(2) Anion exchange membrane was Ionics, Inc., Nepton AR111A, 0.024 inch thick.

(3) Cation exchange membrane was Ionics, Inc., Nepton Cr-61, 0.024 inch thick.

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